



TITLE OF THE INVENTION

HUMAN N-METHYL-D-ASPARTATE RECEPTOR SUBUNITS, NUCLEIC ACIDS ENCODING SAME AND USES THEREFOR

5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of United States Serial No. 08/052,449, filed April 20, 1993, now pending.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

10 Not applicable.

REFERENCE TO MICROFICHE APPENDIX

Not applicable.

15 FIELD OF THE INVENTION

The present invention relates to nucleic acids and receptor proteins encoded thereby. Invention nucleic acids encode novel human N-methyl-D-aspartate (NMDA) receptor subunits. The invention also relates to methods for making such receptor subunits and for using the receptor proteins in assays designed to identify and 20 characterize compounds which affect the function of such receptors, e.g., agonists and antagonists of NMDA receptors.

BACKGROUND OF THE INVENTION

The amino acid L-glutamate is a major excitatory 25 neurotransmitter in the mammalian central nervous system. Anatomical, biochemical and electrophysiological analyses suggest that glutamatergic systems are involved in a broad array of neuronal processes, including fast excitatory synaptic transmission, regulation of neurotransmitter releases, long-term potentiation, learning and memory, developmental synaptic plasticity, hypoxic-ischemic damage and neuronal cell death, 30 epileptiform seizures, as well as the pathogenesis of several neurodegenerative disorders. See generally, Monaghan et al., Ann. Rev. Pharmacol. Toxicol. 29:365-402 (1980). This extensive repertoire of functions, especially those related to learning, neurotoxicity and neuropathology, has stimulated recent attempts to describe and define the mechanisms through which glutamate exerts its effects.

Currently, glutamate receptor classification schemes are based on pharmacological criteria. Glutamate has been observed to mediate its effects through receptors that have been categorized into two main groups: ionotropic and metabotropic. Ionotropic glutamate receptors contain integral cation-specific, ligand-gated ion channels, whereas metabotropic glutamate receptors are G-protein-coupled receptors that transduce extracellular signals via activation of intracellular second messenger systems. Ionotropic receptors are further divided into at least two categories based on the pharmacological and functional properties of the receptors.

The two main types of ionotropic receptors are N-methyl-D-aspartic acid (NMDA) and kainic acid (KA)/ α -amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA), formerly called the quisqualic acid, or QUIS, receptor. While the metabotropic receptors bind to some of the same ligands that bind to ionotropic glutamate receptors, the metabotropic receptors alter synaptic physiology via GTP-binding proteins and second messengers such as cyclic AMP, cyclic GMP, diacylglycerol, inositol 1,4,5-triphosphate and calcium [Gundersen et al., Proc. R. Soc. London Ser. 221:127 (1984); Sladeczek et al., Nature 317:717 (1985); Nicoletti et al., J. Neurosci. 6:1905 (1986); Sugiyama et al., Nature 325:531 (1987)].

The electrophysiological and pharmacological properties of the glutamate receptors have been studied using animal tissues and cell lines, as well as recombinantly produced non-human receptors, as the source of such receptors. The value of such studies for application to the development of human therapeutics has been limited by the availability of only non-human receptor subunits. Moreover, it is only recently that the characteristics and structure of glutamate receptors have been investigated at the molecular level. The majority of such investigation has, however, been carried out in non-human species. Because of the potential physiological and pathological significance of glutamate receptors, it would be desirable (for example, for drug screening assays) to have available human sequences (i.e., DNA, RNA, proteins) which encode representative members of the various glutamate receptor subtypes. The availability of such human sequences will also enable the investigation of receptor distribution in humans, the correlation of specific receptor modification with the occurrence of various disease states, etc.

SUMMARY OF THE INVENTION

The present invention discloses novel nucleic acids encoding NMDA receptor protein subunits and the proteins encoded thereby. In a particular

embodiment the novel nucleic acids encode NMDAR1 and NMDAR2 subunits of human NMDA receptors. More specifically, the invention nucleic acids encode NMDAR1, NMDAR2A, NMDAR2B, NMDAR2C and NMDAR2D subunits that contribute to the formation of NMDA-activated cation-selective ion channels. In addition to being useful for the production of NMDA receptor subunit proteins, these nucleic acids are also useful as probes, thus enabling those skilled in the art, without undue experimentation, to identify and isolate nucleic acids encoding related receptor subunits.

Functional glutamate receptors can be assembled, in accordance with the present invention, from a plurality of NMDA receptor subunit proteins of one type (homomeric) or from combinations of subunit proteins of different types (heteromeric).

In addition to disclosing novel NMDA receptor protein subunits, the present invention also comprises methods for using such receptor subunits to identify and characterize compounds which affect the function of such receptors, e.g., agonists, antagonists, and modulators of glutamate receptor function. The invention also comprises methods for determining whether unknown protein(s) are functional as NMDA receptor subunits.

20 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation of various human NMDAR1 clones of the invention, with partial restriction maps of each clone. The clones are aligned and the differences in the DNAs (i.e., deletions and insertions), relative to clone NMDA10, are indicated. Translation initiation and termination sites are represented by a "V" and a "*", respectively. Insertions are marked as inverted triangles, deletions are indicated by spaces in the boxes. The numbers above the insertions and deletions refer to the number of nucleotides inserted or deleted relative to NMDA10.

Figure 2 is a schematic representation of cDNAs encoding full-length human NMDAR1 subunit subtypes of the invention, with partial restriction maps of each DNA. The full-length cDNAs are constructed by ligation of appropriate portions of the clones shown in Figure 1. Regions of each full-length cDNA composed of nucleotide sequences corresponding to a particular clone are distinguished as solid, striped, cross-hatched or open boxes.

Figure 3 presents the entire nucleotide sequence of construct NMDAR1A (see Sequence ID No. 1) with the following information added for ease of comparison of the splice variations of the NMDAR1 subunit transcript: lowercase letters indicate 5' untranslated sequence and the 3' untranslated sequence of the
5 NMDAR1 splice variant shown in Sequence ID No. 1 (in some of the other splice variants, this 3' untranslated sequence is actually coding sequence); uppercase letters indicate coding sequence; the translation initiation codon is identified by the word "START" whereas the three different translation termination codons (TGA) used in the different splice variants are identified by small boxes; significant restriction
10 enzyme sites used in preparing full-length variant constructs are identified by name above the sites; the location of a 63-bp insertion (see Sequence ID No. 3) that exists in some of the variants is marked as "63 bp INSERT"; the nucleotide sequences that are deleted from some of the variants are boxed and labeled as "204 bp DELETION," "363 bp DELETION," and "1087 bp DELETION."

15 Figure 4 is a schematic representation of various human NMDAR2C clones of the invention, with partial restriction maps of each clone. The clones are aligned and the differences in the DNAs relative to clone NMDA26 are indicated in the same manner as done in Figure 1.

Figure 5 is a schematic representation of full-length human
20 NMDAR2C subunit subtypes of the invention, with partial restriction maps of each DNA. The full-length cDNAs are constructed by ligation of appropriate portions of the clones shown in Figure 4. Regions of each full-length cDNA composed of nucleotide sequences corresponding to a particular clone are distinguished as solid, striped, cross-hatched or open boxes.

25 Figure 6 presents restriction maps of CMV promoter-based vectors pCMV-T7-2 and pCMV-T7-3.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided isolated
30 nucleic acids encoding human N-methyl-D-aspartate (NMDA) receptor subunit(s). In one aspect of the present invention, nucleic acids encoding NMDA receptor subunit(s) of the NMDAR1 subtype are provided. In another aspect, nucleic acids encoding NMDA receptor subunit(s) of the NMDAR2 subtype are provided. In a further aspect, eukaryotic cells containing such nucleic acids, and eukaryotic cells expressing
35 such nucleic acids are provided.

Also provided are protein(s) encoded by the above-described nucleic acids, as well as antibodies generated against the protein(s). In other aspects of the present invention, there are provided nucleic acid probes comprising at least NMDA receptor subunit-selective portions of the above-described nucleic acids.

- 5 As employed herein, the phrase "human N-methyl-D-aspartate (NMDA) receptor subunit(s)" refers to recombinantly produced (i.e., isolated or substantially pure) proteins which participate in the formation of a voltage-sensitive cation-selective channel activated by exposure to NMDA, and having at least one transmembrane domain, a large N-terminal extracellular domain, and the like,
- 10 including variants thereof encoded by mRNA generated by alternative splicing of a primary transcript, and further including fragments thereof which retain one or more of the above properties.

- Use of the phrase "recombinantly produced", "isolated" or "substantially pure" in the present specification and claims as a modifier of DNA, RNA, polypeptides or proteins means that the DNA, RNA, polypeptides or proteins so designated have been produced in such form by the hand of man, and thus are separated from their native *in vivo* cellular environment. As a result of this human intervention, the recombinant DNAs, RNAs, polypeptides and proteins of the invention are useful in ways that the DNAs, RNAs, polypeptides or proteins as they naturally occur are not, such as identification of selective drugs or compounds.

The term "functional", when used herein as a modifier of receptor protein(s) of the present invention, means that binding of NMDA (or NMDA-like) ligand to receptors comprising the protein(s) causes the receptor "ion channels" to open. This allows cations, particularly Ca²⁺, as well as Na⁺ and K⁺, to move across the membrane. Stated another way, "functional" means that a signal is generated as a consequence of agonist activation of receptor protein(s).

As used herein, a splice variant refers to variant NMDA receptor subunit-encoding nucleic acid(s) produced by differential processing of primary transcript(s) of genomic DNA, resulting in the production of more than one type of mRNA. cDNA derived from differentially processed primary transcript will encode NMDA receptor subunits that have regions of complete amino acid identity and regions having different amino acid sequences. Thus, the same genomic sequence can lead to the production of multiple, related mRNAs and proteins. Both the resulting mRNAs and proteins are referred to herein as "splice variants".

Accordingly, also contemplated within the scope of the present invention are DNAs that encode NMDA receptor subunits as defined above, but that by virtue of degeneracy of the genetic code do not necessarily hybridize to the disclosed DNA under specified hybridization conditions. Such subunits also 5 contribute to the formation of functional receptor, as assessed by methods described herein or known to those of skill in the art, with one or more additional NMDA receptor subunits of the same or different type (the presence of additional subunits of a different type is optional when said subunit is an NMDAR1 subunit). Typically, unless an NMDA receptor subunit is encoded by RNA that arises from alternative 10 splicing (i.e., a splice variant), NMDA receptor subunit-encoding DNA and the NMDA receptor subunit encoded thereby share substantial sequence homology with at least one of the NMDA receptor subunit DNAs (and proteins encoded thereby) described herein. It is understood that DNA or RNA encoding a splice variant may share less than 90% overall sequence homology with the DNA or RNA provided 15 herein, but include regions of nearly 100% homology to a DNA fragment described herein, and encode an open reading frame that includes start and stop codons and encodes a functional NMDA receptor subunit.

As employed herein, the phrase "NMDA receptor subunit(s) of the NMDAR1 subtype" refers to proteins which, by hydrophobicity analysis of deduced 20 amino acid sequences, are believed to contain four or more putative transmembrane domains, preceded by a large extracellular N-terminal domain. The amino acid sequence typically contains possible phosphorylation sites for Ca²⁺/calmodulin-dependent protein kinase type II and protein kinase C [see, for example, Kemp et al. (1990) Trends in Biological Science Vol. 15:342-346; Kishimoto et al. (1985) J. Biol. 25 Chem. Vol. 260:12492-12499; Whittemore et al. (1993) Nature 364:70-73]. (These protein kinases reportedly play a crucial role in induction and maintenance of long term potentiation.)

The putative TMII segment (i.e., second transmembrane domain) is typically flanked by a glutamic acid residue at the extracellular side and a stretch of 30 glutamic acid residues at the cytoplasmic side. This segment contains an asparagine residue believed to be responsible for high Ca²⁺ permeability of the NMDAR channel. For a summary of NMDAR properties, see Ben-Ari et al., in TINS 15:333-339 (1992), especially at p. 334.

Exemplary DNA sequences encoding human NMDAR1 subunits are 35 represented by nucleotides which encode substantially the same amino acid sequence

as set forth in Sequence ID Nos. 2, 2E, 2F, 2G, 2H, 2I, 2J, 2K, 2L, 2M, 2N, or 2P. Presently preferred sequences encode substantially the same amino acid sequence as set forth in Sequence ID Nos. 2, 2E, 2F, 2G, 2H, 2I or 2P.

Exemplary DNA can alternatively be characterized as those nucleotide
5 sequences which encode a human NMDAR1 subunit and hybridize under high
stringency conditions to substantially the entire sequence of any one of Sequence ID
Nos. 1, 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1H, 1I, 1J, 1K, 1L, 1M, 1N, or 1P, or substantial
portions thereof (i.e., typically at least 25-30 nucleotides thereof); preferably
exemplary DNA will hybridize under high stringency conditions to substantially the
10 entire sequence of any one of Sequence ID Nos. 1, 1E, 1F, 1G, 1H, 1I or 1P, or
substantial portions thereof.

Stringency of hybridization is used herein to refer to conditions under
which polynucleic acid hybrids are stable. As known to those of skill in the art, the
stability of hybrids is reflected in the melting temperature (T_m) of the hybrids. T_m
15 can be approximated by the formula:

$$81.5^{\circ}\text{C} - 16.6(\log_{10}[\text{Na}^+]) + 0.41(\%G+C) - 600/l,$$

where l is the length of the hybrids in nucleotides. T_m decreases approximately
20 1-1.5°C with every 1% decrease in sequence homology. In general, the stability of a
hybrid is a function of sodium ion concentration and temperature. Typically, the
hybridization reaction is performed under conditions of lower stringency, followed by
washes of varying, but higher, stringency. Reference to hybridization stringency
relates to such washing conditions. Thus, as used herein:
25

(1) HIGH STRINGENCY conditions, with respect to fragment
hybridization, refers to conditions that permit hybridization of
only those nucleic acid sequences that form stable hybrids in
0.018M NaCl at 65°C (i.e., if a hybrid is not stable in 0.018M
NaCl at 65°C, it will not be stable under high stringency
30 conditions, as contemplated herein). High stringency
conditions can be provided, for example, by hybridization in
50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at
42°C, followed by washing in 0.1X SSPE, and 0.1% SDS at
35 65°C;

- (2) MODERATE STRINGENCY conditions, with respect to fragment hybridization, refers to conditions equivalent to hybridization in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at 42°C, followed by washing in 0.2X SSPE, 0.2% SDS, at 65°C;
- 5 (3) LOW STRINGENCY conditions, with respect to fragment hybridization, refers to conditions equivalent to hybridization in 10% formamide, 5X Denhart's solution, 6X SSPE, 0.2% SDS at 42°C, followed by washing in 1X SSPE, 0.2% SDS, at 50°C; and
- 10 (4) HIGH STRINGENCY conditions, with respect to oligonucleotide (i.e., synthetic DNA ≤ about 30 nucleotides in length) hybridization, refers to conditions equivalent to hybridization in 10% formamide, 5X Denhart's solution, 6X SSPE, 0.2% SDS at 42°C, followed by washing in 1X SSPE, and 0.2% SDS at 50°C.
- 15

It is understood that these conditions may be duplicated using a variety of buffers and temperatures and that they are not necessarily precise.

20 Denhart's solution and SSPE (see, e.g., Sambrook, Fritsch, and Maniatis, in: Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989) are well known to those of skill in the art as are other suitable hybridization buffers. For example, SSPE is pH 7.4 phosphate-buffered 0.18M NaCl. SSPE can be prepared, for example, as a 20X stock solution by dissolving 175.3 g of
25 NaCl, 27.6 g of NaH₂PO₄ and 7.4 g EDTA in 800 ml of water, adjusting the pH to 7.4, and then adding water to 1 liter. Denhart's solution (see, Denhart (1966) Biochem. Biophys. Res. Commun. 23:641) can be prepared, for example, as a 50X stock solution by mixing 5 g Ficoll (Type 400, Pharmacia LKB Biotechnology, INC., Piscataway, NJ), 5 g of polyvinylpyrrolidone, 5 g bovine serum albumin (Fraction V;
30 Sigma, St. Louis, MO) water to 500 ml and filtering to remove particulate matter.

Especially preferred sequences are those which have substantially the same nucleotide sequence as the coding sequences in any one of Sequence ID Nos. 1, 1E, 1F, 1G, 1H, 1I, 1J, 1K, 1L, 1M, 1N, or 1P; with those having substantially the same sequence as the coding sequence in Sequence ID Nos. 1, 1E, 1F, 1G, 1H, 1I or
35 1P being most preferred.

As used herein, the phrase "substantial sequence homology" refers to nucleotide sequences which share at least about 90% identity, and amino acid sequences which typically share more than 95% amino acid identity (>99% amino acid identity when dealing with NMDAR1 subunits). It is recognized, however, that 5 proteins (and DNA or mRNA encoding such proteins) containing less than the above-described level of homology arising as splice variants or that are modified by conservative amino acid substitutions (or substitution of degenerate codons) are contemplated to be within the scope of the present invention.

As used herein, the phrase "substantially the same" refers to the 10 nucleotide sequences of DNA, the ribonucleotide sequences of RNA, or the amino acid sequences of protein, that have slight and non-consequential sequence variations from the actual sequences disclosed herein. Species that are "substantially the same" are considered to be equivalent to the disclosed sequences, and as such are within the scope of the appended claims. In this regard, "slight and non-consequential sequence 15 variations" mean that sequences that are substantially the same as the DNA, RNA, or proteins disclosed and claimed herein, are functionally equivalent to the human-derived sequences disclosed and claimed herein. Functionally equivalent sequences will function in substantially the same manner to produce substantially the same compositions as the human-derived nucleic acid and amino acid compositions 20 disclosed and claimed herein. In particular, functionally equivalent DNAs encode human-derived proteins that are the same as those disclosed herein or that have conservative amino acid variations, such as substitution of a non-polar residue for another non-polar residue or a charged residue for a similarly charged residue. These changes include those recognized by those of skill in the art as those that do not 25 substantially alter the tertiary structure of the protein.

As employed herein, the phrase "NMDA receptor subunit(s) of the NMDAR2 subtype" refers to proteins which have a large putative extracellular domain at the amino-terminal region. Otherwise, the deduced structure of NMDAR2 subunits displays the same general characteristics as the NMDAR1 subunit structure. 30 A notable typical exception is that the negatively charged glutamic acid residues that are generally present in the putative TMII segment of NMDAR1 subunits are generally absent from the TMII segment of NMDAR2. Instead, NMDAR2 subunits may contain a positively charged lysine residue in TMII. Unlike NMDAR1 subunits, NMDAR2 subunits generally do not form homomeric NMDA receptors. Moreover,

the amino acid sequences of NMDAR1 and NMDAR2 subunits are generally less than 50% identical, with identities of less than 30% typically observed.

NMDAR2 subunits contemplated by the present invention include NMDAR2A, NMDAR2B, NMDAR2C and NMDAR2D types of subunits.

- 5 Exemplary DNA sequences encoding human NMDAR2A subunits, or portions thereof, are represented by nucleotides which encode substantially the same amino acid sequence as set forth in Sequence ID No. 11, or substantially the same amino acid sequence as that encoded by the NMDAR2A-encoding portion of clone NMDA57, deposited with the ATCC under accession number 75442.

- 10 The deposited clone has been deposited at the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland, U.S.A. 20852, under the terms of the Budapest Treaty on the International Recognition of Deposits of Microorganisms for Purposes of Patent Procedure and the Regulations promulgated under this Treaty. Samples of the deposited material are and will be available to
15 industrial property offices and other persons legally entitled to receive them under the terms of the Treaty and Regulations and otherwise in compliance with the patent laws and regulations of the United States of America and all other nations or international organizations in which this application, or an application claiming priority of this application, is filed or in which any patent granted on any such application is granted.
20 In particular, upon issuance of a U.S. patent based on this or any application claiming priority to or incorporating this application by reference thereto, all restriction upon availability of the deposited material will be irrevocably removed.

- Exemplary human NMDAR2A subunit-encoding DNAs can alternatively be characterized as those nucleotide sequences which hybridize under
25 high stringency conditions to substantially the entire sequence of Sequence ID No. 10, or substantial portions thereof (i.e., typically at least 25-30 nucleotides thereof), or the NMDAR2A-encoding portion of clone NMDA57 (ATCC accession No. 75442). Especially preferred sequences encoding human NMDAR2A subunits are those which have substantially the same nucleotide sequence as the coding sequence of Sequence
30 ID No. 10, or those which contain substantially the same nucleotide sequence as the coding sequence in the NMDAR2A-encoding portion of clone NMDA57.

- Exemplary DNA sequences encoding human NMDAR2B subunits are represented by nucleotides which encode substantially the same amino acid sequence as set forth in Sequence ID No. 14. Exemplary DNAs can alternatively be
35 characterized as those nucleotide sequences which encode a human NMDAR2B

subunit and hybridize under high stringency conditions to substantially the entire sequence of Sequence ID No. 13, or substantial portions thereof (i.e., typically at least 25-30 nucleotides thereof). Especially preferred NMDAR2B-encoding sequences are those which have substantially the same nucleotide sequence as the coding sequence 5 in Sequence ID No. 13.

Exemplary DNA sequences encoding human NMDAR2C subunits are represented by nucleotides which encode substantially the same amino acid sequence as set forth in Sequence ID Nos. 6, 6E, 6F, 6G, 6H or 6I.

Exemplary DNAs can alternatively be characterized as those 10 nucleotide sequences which encode a human NMDAR2C subunit and hybridize under high stringency conditions to substantially the entire sequence of any one of Sequence ID Nos. 5, 5A, 5B, 5C, 5D, 5E, 5F, 5G, 5H, or 5I, or substantial portions thereof (i.e., typically at least 25-30 nucleotides thereof); preferably exemplary DNA will hybridize under high stringency conditions to substantially the entire sequence of any 15 one of Sequence ID Nos. 5, 5E, 5F, or 5G, or substantial portions thereof.

Especially preferred NMDAR2C-encoding sequences are those which have substantially the same nucleotide sequence as the coding sequences in any one of Sequence ID Nos. 5, 5E, 5F, 5G, 5H or 5I; with those having substantially the same sequence as the coding sequences in Sequence ID Nos. 5, 5E, 5F, or 5G being most 20 preferred.

Exemplary DNA sequences encoding human NMDAR2D subunits are represented by nucleotides which encode substantially the same amino acid sequence as set forth in Sequence ID No. 16. Exemplary DNAs can alternatively be characterized as those nucleotide sequences which encode a human NMDAR2D 25 subunit and hybridize under high stringency conditions to substantially the entire sequence of Sequence ID No. 15, or substantial portions thereof (i.e., typically at least 25-30 nucleotides thereof). Especially preferred NMDAR2D-encoding sequences are those which have substantially the same nucleotide sequence as the coding sequence in Sequence ID No. 15.

30 DNA encoding human NMDA receptor subunits may be isolated by screening suitable human cDNA or human genomic libraries under suitable hybridization conditions with DNA disclosed herein (including nucleotides derived from any of SEQ ID Nos. 1, 1A-1P, 5, 5A-5I, 10, 13 or 15). Suitable libraries can be prepared from neuronal tissue samples, e.g., hippocampus and cerebellum tissue, cell 35 lines, and the like. For example, the library can be screened with a portion of DNA

including substantially the entire subunit-encoding sequence thereof, or the library may be screened with a suitable probe.

As used herein, a probe is single-stranded DNA or RNA that has a sequence of nucleotides that includes at least 14 contiguous bases that are the same as 5 (or the complement of) any 14 or more contiguous bases set forth in any of SEQ ID Nos. 1, 1A-1P, 5, 5A-5I, 10, 13 or 15. Preferred regions from which to construct probes include 5' and/or 3' coding sequences, sequences predicted to encode transmembrane domains, sequences predicted to encode cytoplasmic loops, signal sequences, NMDA binding sites, and the like.

10 Either the full-length cDNA clones or fragments thereof can be used as probes, preferably labeled with suitable label means for ready detection. When fragments are used as probes, preferably the DNA sequences will be from the carboxyl end-encoding portion of the DNA, and most preferably will include predicted transmembrane domain-encoding portions of the DNA sequence (the 15 domains can be predicted based on hydropathy analysis of the deduced amino acid sequence using, for example, the method of Kyte and Doolittle (1982), *J. Mol. Biol.* Vol. 157:105). These probes can be used, for example, for the identification and isolation of additional members of the glutamate receptor family.

As a particular application of the invention sequences, genetic
20 screening can be carried out using the nucleotide sequences of the invention as probes. Thus, nucleic acid samples from patients having neuropathological conditions suspected of involving alteration/modification of any one or more of the glutamate receptors can be screened with appropriate probes to determine if any abnormalities exist with respect to any of the endogenous glutamate receptors. Similarly, patients
25 having a family history of disease states related to glutamate receptor dysfunction can be screened to determine if they are also predisposed to such disease states.

In accordance with another embodiment of the present invention, there is provided a method for identifying DNA encoding human N-methyl-D-aspartate (NMDA) receptor protein subunit(s), said method comprising:

30 contacting human DNA with a nucleic acid probe as described above, wherein said contacting is carried out under high stringency hybridization conditions, and

identifying DNA(s) which hybridize to said probe.

After screening the library, positive clones are identified by detecting a
35 hybridization signal; the identified clones are characterized by restriction enzyme

mapping and/or DNA sequence analysis, and then examined by comparison with the sequences set forth herein to ascertain whether they include DNA encoding a complete NMDA receptor subunit (i.e., if they include translation initiation and termination codons). If the selected clones are incomplete, they may be used to

5 rescreen the same or a different library to obtain overlapping clones. If the library is genomic, then the overlapping clones may include exons and introns. If the library is a cDNA library, then the overlapping clones will include an open reading frame. In both instances, complete clones may be identified by comparison with the DNA and encoded proteins provided herein.

10 Complementary DNA clones encoding various human NMDA receptor subunits (e.g., NMDAR1, NMDAR2A, NMDAR2B, NMDAR2C, NMDAR2D) have been isolated. Each type of subunit appears to be encoded by a different gene. The DNA clones provided herein may be used to isolate genomic clones encoding each type of subunit and to isolate any splice variants by screening libraries prepared from

15 different neural tissues. Nucleic acid amplification techniques, which are well known in the art, can be used to locate DNA encoding splice variants of human NMDA receptor subunits. This is accomplished by employing oligonucleotides based on DNA sequences surrounding divergent sequence(s) as primers for amplifying human RNA or genomic DNA. Size and sequence determinations of the amplification

20 products can reveal the existence of splice variants. Furthermore, isolation of human genomic DNA sequences by hybridization can yield DNA containing multiple exons, separated by introns, that correspond to different splice variants of transcripts encoding human NMDA receptor subunits.

It has been found that not all subunits (and variants thereof) are

25 expressed in all neural tissues or in all portions of the brain. Thus, in order to isolate cDNA encoding a particular subunit or splice variants thereof, it is preferable to screen libraries prepared from different neuronal or neural tissues. Preferred tissues to use as sources of nucleic acids for preparing libraries to obtain DNA encoding each subunit include: hippocampus to isolate human NMDAR1-encoding DNAs;

30 hippocampus, cerebellum and fetal brain to isolate NMDAR2-encoding DNAs; and the like.

Once DNA encoding a subunit has been isolated, ribonuclease (RNase) protection assays can be employed to determine which tissues express mRNA encoding a particular NMDAR subunit subtype or variant. These assays provide a

35 sensitive means for detecting and quantitating an RNA species in a complex mixture

of total cellular RNA. The subunit DNA is labeled and hybridized with cellular RNA. If complementary mRNA is present in the cellular RNA, a DNA-RNA hybrid results. The RNA sample is then treated with RNase, which degrades single-stranded RNA. Any RNA-DNA hybrids are protected from RNase degradation and can be visualized 5 by gel electrophoresis and autoradiography. *In situ* hybridization techniques can also be used to determine which tissues express mRNA encoding a particular NMDAR subunit. The labeled subunit DNAs are hybridized to different brain region slices to visualize subunit mRNA expression.

The distribution of expression of some human NMDA receptor
10 subunits may differ from the distribution of such receptors in rat. For example, RNA encoding the rat NMDAR2C subunit is abundant in rat cerebellum, but is not abundant in rat hippocampus [see, e.g., Monyer et al., Science 256:1217-1221 (1992)]. Numerous human NMDAR2C clones were ultimately obtained, however, from a human hippocampus library. Thus, the distribution of some NMDA receptor
15 subunits in humans and rats appears to be different.

The above-described nucleotide sequences can be incorporated into vectors for further manipulation. As used herein, vector (or plasmid) refers to discrete elements that are used to introduce heterologous DNA into cells for either expression or replication thereof. Selection and use of such vehicles are well within the skill of
20 the artisan.

An expression vector includes vectors capable of expressing DNAs that are operatively linked with regulatory sequences, such as promoter regions, that are capable of effecting expression of such DNA fragments. Thus, an expression vector refers to a recombinant DNA or RNA construct, such as a plasmid, a phage,
25 recombinant virus or other vector that, upon introduction into an appropriate host cell, results in expression of the cloned DNA. Appropriate expression vectors are well known to those of skill in the art and include those that are replicable in eukaryotic cells and/or prokaryotic cells and those that remain episomal or those which integrate into the host cell genome. Presently preferred plasmids for expression of invention
30 NMDA receptor subunits in eukaryotic host cells, particularly mammalian cells, include cytomegalovirus (CMV) promoter-containing vectors such as pCMV-T7-2 or pCMV-T7-3 (see Figure 6), pMMTVT7(+) or pMMTVT7(-) (modified versions of pMAMneo (Clontech, Palo Alto, CA), prepared as described herein), pcDNA1, and the like.

As used herein, a promoter region refers to a segment of DNA that controls transcription of DNA to which it is operatively linked. The promoter region includes specific sequences that are sufficient for RNA polymerase recognition, binding and transcription initiation. This portion of the promoter region is referred to 5 as the promoter. In addition, the promoter region includes sequences that modulate this recognition, binding and transcription initiation activity of RNA polymerase. These sequences may be *cis* acting or may be responsive to *trans* acting factors. Promoters, depending upon the nature of the regulation, may be constitutive or regulated. Exemplary promoters contemplated for use in the practice of the present 10 invention include the SV40 early promoter, the cytomegalovirus (CMV) promoter, the mouse mammary tumor virus (MMTV) steroid-inducible promoter, Moloney murine leukemia virus (MMLV) promoter, and the like.

As used herein, the term "operatively linked" refers to the functional relationship of DNA with regulatory and effector sequences of nucleotides, such as 15 promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes and binds to the promoter, and transcribes the DNA. In order 20 to optimize expression and/or *in vitro* transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation (i.e., start) codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, for example, Kozak 25 (1991) *J. Biol. Chem.* 266:19867-19870) can be inserted immediately 5' of the start codon and may enhance expression. Likewise, alternative codons, encoding the same amino acid, can be substituted for coding sequences of the NMDAR subunits in order to enhance transcription (e.g., the codon preference of the host cells can be adopted, the presence of G-C rich domains can be reduced, and the like). Furthermore, for 30 potentially enhanced expression of NMDA receptor subunits in amphibian oocytes, the subunit coding sequence can optionally be incorporated into an expression construct wherein the 5'- and 3'-ends of the coding sequence are contiguous with *Xenopus* β-globin gene 5' and 3' untranslated sequences, respectively. For example, NMDA receptor subunit coding sequences can be incorporated into vector pSP64T 35 (see Krieg and Melton (1984) in *Nucleic Acids Research* 12:7057-7070), a modified

form of pSP64 (available from Promega, Madison, WI). The coding sequence is inserted between the 5' end of the β -globin gene and the 3' untranslated sequences located downstream of the SP6 promoter. *In vitro* transcripts can then be generated from the resulting vector. The desirability of (or need for) such modification may be 5 empirically determined.

As used herein, expression refers to the process by which polynucleic acids are transcribed into mRNA and translated into peptides, polypeptides, or proteins. If the polynucleic acid is derived from genomic DNA, expression may, if an appropriate eukaryotic host cell or organism is selected, include splicing of the 10 mRNA.

Particularly preferred vectors for transfection of mammalian cells are the pSV2dhfr expression vectors, which contain the SV40 early promoter, mouse dhfr gene, SV40 polyadenylation and splice sites and sequences necessary for maintaining the vector in bacteria, cytomegalovirus (CMV) promoter-based vectors such as 15 pCMV-T7-2 and pCMV-T7-3 (described herein) or pCDNA1 (Invitrogen, San Diego, CA), and MMTV promoter-based vectors such as pMMTVT7(+) or pMMTVT7(-), described herein.

Full-length DNAs encoding human NMDA receptor subunits have been inserted into vectors pcDNA1, pMMTVT7(+), pCMV-T7-2 and pCMV-T7-3. 20 pCMV-T7-2 is a pUC19-based mammalian cell expression vector containing the CMV promoter/enhancer, SV40 splice/donor sites located immediately downstream of the promoter, a T7 bacteriophage RNA polymerase promoter positioned downstream of the splice sites, followed by an SV40 polyadenylation signal and a polylinker between the T7 promoter and the polyadenylation signal. Placement of 25 NMDA receptor subunit DNA between the CMV promoter and SV40 polyadenylation signal should provide for constitutive expression of the foreign DNA in a mammalian host cell transfected with the construct. Plasmid pCMV-T7-3 is identical to pCMV-T7-2 except that the order of restriction enzyme sites in the polylinker is reversed.

Vectors pMMTVT7(+) and pMMTVT7(-) were prepared by modifying 30 vector pMAMneo (Clontech, Palo Alto, CA). pMAMneo is a mammalian expression vector that contains the Rous Sarcoma Virus (RSV) long terminal repeat (LTR) enhancer, linked to the dexamethasone-inducible mouse mammary tumor virus (MMTV)-LTR promoter, followed by SV40 splicing and polyadenylation sites. pMAMneo also contains the *E. coli* neo gene for selection of transformants, as well as

the β -lactamase gene (encoding a protein which imparts ampicillin-resistance) for propagation in *E. coli*.

Vector pMMTVT7(+) can be generated by modification of pMAMneo to remove the *neo* gene and insert the multiple cloning site and T7 and T3 promoters from pBluescript (Stratagene, La Jolla, CA). Thus, pMMTVT7(+) contains the RSV-LTR enhancer linked to the MMTV-LTR promoter, a T7 bacteriophage RNA polymerase promoter positioned downstream of the MMTV-LTR promoter, a polylinker positioned downstream of the T7 promoter, a T3 bacteriophage RNA polymerase promoter positioned downstream of the T7 promoter, and SV40 splicing 10 and polyadenylation sites positioned downstream of the T3 promoter. The β -lactamase gene (encoding a protein which imparts ampicillin-resistance) from pMAMneo is retained in pMMTVT7(+), although it is incorporated in the reverse orientation relative to the orientation in pMAMneo.

Vector pMMTVT7(-) is identical to pMMTVT7(+) except that the 15 positions of the T7 and T3 promoters are switched, i.e., the T3 promoter in pMMTVT7(-) is located where the T7 promoter is located in pMMTVT7(+), and the T7 promoter in pMMTVT7(-) is located where the T3 promoter is located in pMMTVT7(+). Therefore, vectors pMMTVT7(+) and pMMTVT7(-) contain all of the regulatory elements required for expression of heterologous DNA in a mammalian 20 host cell, wherein the heterologous DNA has been incorporated into the vectors at the polylinker. In addition, because the T7 and T3 promoters are located on either side of the polylinker, these plasmids can be used for synthesis of *in vitro* transcripts of heterologous DNA that has been subcloned into the vectors at the polylinker.

For inducible expression of human NMDA receptor subunit-encoding 25 DNA in a mammalian cell, the DNA can be inserted into a plasmid such as pMMTVT7(+) or pMMTVT7(-). These plasmids contain the mouse mammary tumor virus (MMTV) promoter for steroid-inducible expression of operatively associated foreign DNA. If the host cell does not express endogenous glucocorticoid receptors required for uptake of glucocorticoids (i.e., inducers of the MMTV promoter) into the 30 cell, it is necessary to additionally transfect the cell with DNA encoding the glucocorticoid receptor (ATCC accession no. 67200). For synthesis of *in vitro* transcripts, full-length human DNA clones encoding human NMDAR1, NMDAR2A, NMDAR2B, NMDAR2C and NMDAR2D can also be subcloned into pIBI24 (International Biotechnologies, Inc., New Haven, CT), pCMV-T7-2, pCMV-T7-3,

pMMT7(+), pMMT7(-), pBluescript (Stratagene, La Jolla, CA) or pGEM7Z (Promega, Madison, WI).

In accordance with another embodiment of the present invention, there are provided cells containing the above-described polynucleic acids (i.e., DNA or mRNA). Such host cells as bacterial, yeast and mammalian cells can be used for replicating DNA and producing NMDA receptor subunit(s). Methods for assessing receptor expression and function are described in PCT Application Nos. 5 PCT/US91/05625 and PCT/US92/11090, and in co-pending U.S. Application Serial Nos. 07/563,751 and 07/812,254. The subject matter of these documents is hereby 10 incorporated by reference herein in their entirety.

Incorporation of cloned DNA into a suitable expression vector, transfection of eukaryotic cells with a plasmid vector or a combination of plasmid vectors, each encoding one or more distinct genes or with linear DNA, and selection of transfected cells are well known in the art (see, e.g., Sambrook et al. (1989)

- 15 15 Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press). Heterologous DNA may be introduced into host cells by any method known to those of skill in the art, such as transfection with a vector encoding the heterologous DNA by CaPO₄ precipitation (see, e.g., Wigler et al. (1979) Proc. Natl. Acad. Sci. 76:1373-1376) or lipofectamine (GIBCO BRL #18324-012).
- 20 20 Recombinant cells can then be cultured under conditions whereby the subunit(s) encoded by the DNA is (are) expressed. Preferred cells include mammalian cells (e.g., HEK293, CHO, BHKBI and Ltk⁻ cells, mouse monocyte macrophage P388D1 and J774A-1 cells (available from ATCC, Rockville, MD), and the like), yeast cells (e.g., methylotrophic yeast cells, such as *Pichia pastoris*), bacterial cells (e.g.,
- 25 25 *Escherichia coli*), and the like.

While the DNA provided herein may be expressed in any eukaryotic cell, including yeast cells (such as, for example, *P. pastoris* (see U.S. Patent Nos. 4,882,279, 4,837,148, 4,929,555 and 4,855,231), *Saccharomyces cerevisiae*, *Candida tropicalis*, *Hansenula polymorpha*, and the like), mammalian expression systems, 30 including commercially available systems and other such systems known to those of skill in the art, for expression of DNA encoding the human NMDA receptor subunits provided herein are presently preferred. *Xenopus* oocytes are preferred for expression of *in vitro* RNA transcripts of the DNA.

In preferred embodiments, human NMDAR subunit-encoding DNA is 35 ligated into a vector, and introduced into suitable host cells to produce transformed

cell lines that express a specific human NMDA receptor subtype, or specific combinations of subunits. The resulting cell lines can then be produced in quantity for reproducible quantitative analysis of the effects of known or potential drugs on receptor function. In other embodiments, mRNA may be produced by *in vitro* transcription of DNA encoding each subunit. This mRNA, either from a single subunit clone or from a combination of clones, can then be injected into *Xenopus* oocytes where the mRNA directs the synthesis of the human receptor subunits, which then form functional receptors. Alternatively, the subunit-encoding DNA can be directly injected into oocytes for expression of functional receptors. The transfected mammalian cells or injected oocytes may then be used in the methods of drug screening provided herein.

Eukaryotic cells in which DNA or RNA may be introduced include any cells that are transfectable by such DNA or RNA or into which such DNA or RNA may be injected. Preferred cells are those that can be transiently or stably transfected and also express the DNA and RNA. Presently most preferred cells are those that can form recombinant or heterologous human NMDA receptors comprising one or more subunits encoded by the heterologous DNA. Such cells may be identified empirically or selected from among those known to be readily transfected or injected.

Exemplary cells for introducing DNA include cells of mammalian origin (e.g., COS cells, mouse L cells, Chinese hamster ovary (CHO) cells, human embryonic kidney (HEK) cells (particularly HEK293 cells that can be frozen in liquid nitrogen and then thawed and regrown; for example, those described in U.S. Patent No. 5,024,939 to Gorman (see, also, Stillman et al. (1985) Mol. Cell. Biol. 5:2051-2060)), African green monkey cells and other such cells known to those of skill in the art), amphibian cells (e.g., *Xenopus laevis* oocytes), yeast cells (e.g., *Saccharomyces cerevisiae*, *Pichia pastoris*), and the like. Exemplary cells for expressing injected RNA transcripts include *Xenopus laevis* oocytes. Cells that are preferred for transfection of DNA are known to those of skill in the art or may be empirically identified, and include HEK293 (which are available from ATCC under accession #CRL 1573); Ltk⁻ cells (which are available from ATCC under accession #CCL1.3); COS-7 cells (which are available from ATCC under accession #CRL 1651); and DG44 cells (dhfr⁻ CHO cells; see, e.g., Urlaub et al. (1986) Cell. Molec. Genet. 12: 555). Presently preferred cells include Ltk⁻ cells and DG44 cells.

DNA may be stably incorporated into cells or may be transiently expressed using methods known in the art. Stably transfected mammalian cells may be prepared by transfecting cells with an expression vector having a selectable marker gene (such as, for example, the gene for thymidine kinase, dihydrofolate reductase, 5 neomycin resistance, and the like), and growing the transfected cells under conditions selective for cells expressing the marker gene. To prepare transient transfectants, mammalian cells are transfected with a reporter gene (such as the *E. coli* β -galactosidase gene) to monitor transfection efficiency. Selectable marker genes are not included in the transient transfections because the transfectants are typically not 10 grown under selective conditions, and are usually analyzed within a few days after transfection.

To produce such stably or transiently transfected cells, the cells should be transfected with a sufficient concentration of subunit-encoding nucleic acids to form human NMDA receptors that contain the human subunits encoded by 15 heterologous DNA. The precise amounts and ratios of DNA encoding the subunits may be empirically determined and optimized for a particular combination of subunits, cells and assay conditions. Recombinant cells that express NMDA receptors containing subunits encoded only by the heterologous DNA or RNA are especially preferred.

20 Heterologous DNA may be maintained in the cell as an episomal element or may be integrated into chromosomal DNA of the cell. The resulting recombinant cells may then be cultured or subcultured (or passaged, in the case of mammalian cells) from such a culture or a subculture thereof. Methods for transfection, injection and culturing recombinant cells are known to the skilled 25 artisan. Similarly, the human NMDA receptor subunits may be purified using protein purification methods known to those of skill in the art. For example, antibodies or other ligands that specifically bind to one or more of the subunits may be used for affinity purification and immunoprecipitation of the subunit or human NMDA receptors containing the subunits.

30 As used herein, heterologous or foreign DNA and RNA are used interchangeably and refer to DNA or RNA that does not occur naturally as part of the genome of the cell in which it is present or to DNA or RNA which is found in a location or locations in the genome that differ from that in which it occurs in nature. Typically, heterologous or foreign DNA and RNA refers to DNA or RNA that is not 35 endogenous to the host cell and has been artificially introduced into the cell.

Examples of heterologous DNA include DNA that encodes a human NMDA receptor subunit, DNA that encodes RNA or proteins that mediate or alter expression of endogenous DNA by affecting transcription, translation, or other regulatable biochemical processes, and the like. The cell that expresses heterologous DNA may 5 contain DNA encoding the same or different expression products. Heterologous DNA need not be expressed and may be integrated into the host cell genome or maintained episomally.

Recombinant receptors on recombinant eukaryotic cell surfaces may contain one or more subunits encoded by the DNA or mRNA encoding human 10 NMDA receptor subunits, or may contain a mixture of subunits encoded by the host cell and subunits encoded by heterologous DNA or mRNA. Recombinant receptors may be homomeric or may be a heteromeric combination of multiple subunits. Mixtures of DNA or mRNA encoding receptors from various species, such as rats and humans, may also be introduced into the cells. Thus, a cell can be prepared that 15 expresses recombinant receptors containing only NMDAR1 subunits, or a combination of any one or more NMDAR1 and any one or more NMDAR2 subunits provided herein. For example, NMDAR1 subunits of the present invention can be co-expressed with NMDAR2A, NMDAR2B, NMDAR2C and/or NMDAR2D receptor subunits. Specific examples of heteromeric combinations of recombinant human 20 NMDAR subunits that have been expressed in *Xenopus* oocytes include NMDAR1 + NMDAR2A, NMDAR1 + NMDAR2B, and NMDAR1 + NMDAR2A + NMDAR2C (see Example 9).

The DNA, mRNA, vectors, receptor subunits, receptor subunit 25 combinations and cells provided herein permit production of selected NMDA receptor subunits and specific combinations thereof, as well as antibodies to said receptor subunits. This provides a means to prepare synthetic or recombinant receptors and receptor subunits that are substantially free of contamination from many other receptor proteins whose presence can interfere with analysis of a single NMDA receptor subtype. The availability of desired receptor subtypes makes it possible to 30 observe the effect of a drug substance on a particular receptor subtype or combination of NMDA receptor subunits, and to thereby perform initial *in vitro* screening of the drug substance in a test system that is specific for humans and specific for a human NMDA receptor subtype or combination of NMDA receptor subunits. The availability of specific antibodies makes it possible to identify the subunit

combinations expressed *in vivo*. Such specific combinations can then be employed as preferred targets in drug screening.

- The ability to screen drug substances *in vitro* to determine the effect of the drug on specific receptor compositions should permit the development and
- 5 screening of receptor subtype-specific or disease-specific drugs. Also, testing of single receptor subunits or specific combinations of various types of receptor subunits with a variety of potential agonists or antagonists provides additional information with respect to the function and activity of the individual subunits and should lead to the identification and design of compounds that are capable of very specific
- 10 interaction with one or more types of receptor subunits or receptor subtypes. The resulting drugs should exhibit fewer unwanted side effects than drugs identified by screening with cells that express a variety of receptor subtypes.

Further in relation to drug development and therapeutic treatment of various disease states, the availability of DNAs encoding human NMDA receptor

15 subunits enables identification of any alterations in such genes (e.g., mutations) which may correlate with the occurrence of certain disease states. In addition, the creation of animal models of such disease states becomes possible, by specifically introducing such mutations into synthetic DNA sequences which can then be introduced into laboratory animals or *in vitro* assay systems to determine the effects thereof.

20 In another aspect, the invention comprises functional peptide fragments, and functional combinations thereof, encoded by the DNAs of the invention. Such functional peptide fragments can be produced by those skilled in the art, without undue experimentation, by eliminating some or all of the amino acids in the sequence not essential for the peptide to function as a glutamate receptor. A

25 determination of the amino acids that are essential for glutamate receptor function is made, for example, by systematic digestion of the DNAs encoding the peptides and/or by the introduction of deletions into the DNAs. The modified (e.g., deleted or digested) DNAs are expressed, for example, by transcribing the DNA and then introducing the resulting mRNA into *Xenopus* oocytes, where translation of the

30 mRNAs will occur. Functional analysis of the proteins thus expressed in the oocytes is accomplished by exposing the oocytes to ligands known to bind to and functionally activate glutamate receptors, and then monitoring the oocytes to see if the expressed fragments form ion channel(s). If ion channel(s) are detected, the fragments are functional as glutamate receptors.

The above-described method can be carried out in the presence of NMDAR1-like receptor subunits alone, or in the presence of combinations of NMDAR1-like and NMDAR2-like receptor subunits. Thus, for example, when the protein being tested is an NMDAR2-like receptor subunit, the additional subunit is 5 preferably an NMDAR1-like subunit.

In accordance with still another embodiment of the present invention, there is provided a method for identifying compounds which bind to human N-methyl-D-aspartate (NMDA) receptor subunit(s), said method comprising employing receptor proteins of the invention in a competitive binding assay. Such an 10 assay can accommodate the rapid screening of a large number of compounds to determine which compounds, if any, are capable of binding to NMDA receptors. Subsequently, more detailed assays can be carried out with those compounds found to bind, to further determine whether such compounds act as modulators, agonists or antagonists of invention receptors.

15 Another application of the binding assay of the invention is the assay of test samples (e.g., biological fluids) for the presence or absence of receptors of the present invention. Thus, for example, serum from a patient displaying symptoms related to glutamatergic pathway dysfunction can be assayed to determine if the observed symptoms are perhaps caused by over- or under-production of such 20 receptor(s).

The binding assays contemplated by the present invention can be carried out in a variety of ways, as can readily be identified by those of skill in the art. For example, competitive binding assays can be employed, such as radioreceptor assays, and the like.

25 In accordance with a further embodiment of the present invention, there is provided a bioassay for identifying compounds which modulate the activity of human NMDA receptors of the invention, said bioassay comprising:

- 30 (a) exposing cells containing DNA encoding human NMDA receptor subunit(s), wherein said cells express functional NMDA receptors, to at least one compound whose ability to modulate the ion channel activity of said receptors is sought to be determined; and thereafter
- (b) monitoring said cells for changes in ion channel activity.

The above-described bioassay enables the identification of agonists 35 and antagonists for human NMDA receptors. According to this method, recombinant

NMDA receptors are contacted with an "unknown" or test substance (in the further presence of a known NMDA agonist, when antagonist activity is being tested), the ion channel activity of the known glutamate receptor is monitored subsequent to the contact with the "unknown" or test substance, and those substances which increase or decrease the ion channel response of the known glutamate receptor(s) are identified as functional ligands (i.e., modulators, agonists or antagonists) for human NMDA receptors.

In accordance with a particular embodiment of the present invention, recombinant human NMDA receptor-expressing mammalian cells or oocytes can be contacted with a test compound, and the modulating effect(s) thereof can then be evaluated by comparing the NMDA receptor-mediated response in the presence and absence of test compound, or by comparing the response of test cells, or control cells (i.e., cells that do not express NMDA receptors), to the presence of the compound.

As used herein, a compound or signal that "modulates the activity of an NMDA receptor" refers to a compound or signal that alters the activity of NMDA receptors so that activity of the NMDA receptor is different in the presence of the compound or signal than in the absence of the compound or signal. In particular, such compounds or signals include agonists and antagonists. The term agonist refers to a substance or signal, such as NMDA, that activates receptor function; and the term antagonist refers to a substance that interferes with receptor function. Typically, the effect of an antagonist is observed as a blocking of activation by an agonist.

Antagonists include competitive and non-competitive antagonists. A competitive antagonist (or competitive blocker) interacts with or near the site specific for the agonist (e.g., ligand or neurotransmitter). A non-competitive antagonist or blocker inactivates the functioning of the receptor by interacting with a site other than the site that interacts with the agonist.

As understood by those of skill in the art, assay methods for identifying compounds that modulate human NMDA receptor activity (e.g., agonists and antagonists) generally require comparison to a control. One type of a "control" cell or "control" culture is a cell or culture that is treated substantially the same as the cell or culture exposed to the test compound, except the control culture is not exposed to test compound. For example, in methods that use voltage clamp electrophysiological procedures, the same cell can be tested in the presence and absence of test compound, by merely changing the external solution bathing the cell.

Another type of "control" cell or "control" culture may be a cell or a culture of cells

which is identical to the transfected cells, except the cells employed for the control culture do not express functional human NMDA receptor subunits. In this situation, the response of test cell to test compound is compared to the response (or lack of response) of receptor-negative (control) cell to test compound, when cells or cultures 5 of each type of cell are exposed to substantially the same reaction conditions in the presence of compound being assayed.

In accordance with yet another embodiment of the present invention, the ion channel activity of human N-methyl-D-aspartate (NMDA) receptors can be modulated by contacting such receptors with an effective amount of at least one 10 compound identified by the above-described bioassay.

In accordance with yet another embodiment of the present invention, there are provided antibodies generated against the above-described receptor proteins. Such antibodies can be employed for studying receptor tissue localization, subunit composition, structure of functional domains, as well as in diagnostic applications, 15 therapeutic applications, and the like. Preferably, for therapeutic applications, the antibodies employed will be monoclonal antibodies.

The above-described antibodies can be prepared employing standard techniques, as are well known to those of skill in the art, using the invention receptor proteins or portions thereof as antigens for antibody production. Both anti-peptide 20 and anti-fusion protein antibodies can be used [see, for example, Bahouth et al. (1991) Trends Pharmacol Sci. vol. 12:338-343; Current Protocols in Molecular Biology (Ausubel et al., eds.) John Wiley and Sons, New York (1989)]. Factors to consider in selecting portions of the NMDAR subunits for use as immunogen (as either a synthetic peptide or a recombinantly produced bacterial fusion protein) include 25 antigenicity, accessibility (i.e., extracellular and cytoplasmic domains), uniqueness to the particular subunit, etc.

The availability of subunit-specific antibodies makes possible the application of the technique of immunohistochemistry to monitor the distribution and expression density of various subunits (e.g., in normal vs. diseased brain tissue). Such 30 antibodies could also be employed for diagnostic and therapeutic applications.

In accordance with still another embodiment of the present invention, there are provided methods for modulating the ion channel activity of receptor(s) of the invention by contacting said receptor(s) with an effective amount of the above-described antibodies.

The antibodies of the invention can be administered to a subject employing standard methods, such as, for example, by intraperitoneal, intramuscular, intravenous, or subcutaneous injection, implant or transdermal modes of administration, and the like. One of skill in the art can readily determine dose forms, treatment regiments, etc, depending on the mode of administration employed.

5 The invention will now be described in greater detail by reference to the following non-limiting examples.

EXAMPLE 1

10 Isolation of DNA encoding human NMDA receptor NMDAR1 subunits

A. cDNA Library Screening

RNA isolated from human hippocampus tissue was used as a template for the synthesis of oligo dT-primed and randomly primed, single-stranded cDNA 15 according to standard procedures [see, for example, Maniatis et al. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY]. The single-stranded cDNA was converted to double-stranded cDNA, and *Eco*RI/*Sna*BI/*Xho*I adaptors were added to the ends thereof. The cDNAs were separated by size using agarose gel electrophoresis, and those that were >2.0 kb were 20 ligated into *Eco*RI-digested λgt10 bacteriophage vectors. The resulting cDNA library was amplified by replication of each clone through limited infection of a bacterial host, and stored at -70°C.

The amplified hippocampus oligo dT-primed cDNA library was later retrieved from storage and 1 × 10⁶ recombinants were screened for hybridization to 25 oligonucleotides corresponding to nucleotides 96-128 (SE7) and nucleotides 2576-2609 (SE8) of the rat NMDAR1A receptor cDNA (see Moriyoshi et al. (1991) *Nature* 354:31). Hybridization was performed at 42°C in 6X SSPE, 5X Denhart's solution, 10% formamide, 0.2% SDS and 200 µg/ml herring sperm DNA. Washes were 30 performed in 1X SSPE and 0.2% SDS at 50°C. Hybridizing clones (e.g. NMDA1-3) were identified. These clones hybridized to SE8 but not to SE7.

A randomly primed primary human hippocampus cDNA library (~2 × 10⁵ recombinants prepared by selecting only cDNAs >2.0 kb for inclusion in the library) was screened under the same conditions for hybridization to oligonucleotide

SE8 and an oligonucleotide corresponding to nucleotides 129-141 of the rat NMDAR1A receptor cDNA (SE11). Five hybridizing clones, which hybridized to SE8 and not to SE11, were identified: NMDA5-7 and NMDA10-11.

5 B. Characterization of Clones

The clones were plaque purified and characterized by restriction enzyme mapping and DNA sequence analysis of the inserts. One of the clones, NMDA11 (see Sequence ID No. 1B for a description of a portion of NMDA11), is a 10 full-length cDNA (i.e., it contains translation initiation and termination codons) encoding a complete NMDAR1 subunit. The remaining clones are partial cDNAs. Clones NMDA2, NMDA3 (see Sequence ID No. 1D), NMDA5, NMDA6, NMDA7 (see Sequence ID No. 1C), and NMDA10 (see Sequence ID No. 1A for a description of a portion of NMDA10) contain a translation termination codon but lack nucleotides 15 at the 5' end of the coding sequence.

Characterization of the clones revealed that the isolated cDNAs correspond to different alternatively spliced forms of the human NMDAR1 subunit transcript. The four types of alternate splicing represented by the clones are depicted schematically in Figure 1. Clone NMDA10 (which lacks 5' untranslated sequences as 20 well as 60 nucleotides of the 5' end of the coding sequence) is used as a reference to which the other variants are compared. Clone NMDA11 lacks 363 nucleotides (in the 3' portion of the clone) that are present in NMDA10. This 363-nucleotide deletion does not disrupt the reading frame of the transcript; however, it results in a different 25 termination codon. The last 69 nucleotides of the coding sequence of NMDA11 correspond to 3' untranslated sequence of clone NMDA10 (i.e., nucleotides 3325-3393 of Sequence ID No. 1). Clone NMDA7 lacks the same 363-nucleotide sequence that is deleted from NMDA11; however, NMDA7 further lacks 204 nucleotides at the 30 5' end that are present in NMDA10 and NMDA11. This 204-nucleotide deletion also does not disrupt the reading frame of the transcript. Additionally, NMDA7 contains a 63-nucleotide in-frame insertion at the 5' end relative to NMDA10 and NMDA11.

The last 69 base pairs of the coding sequence of NMDA7 correspond to 3' untranslated sequence of NMDA10 i.e., nucleotides 3325-3393 of Sequence ID No. 1). Clone NMDA3 lacks 1087 base pairs at the 3' end that are present in NMDA10. This 1087-base pair deletion does not disrupt the reading frame of the transcript: 35 however it results in a different termination codon. The last 231 base pairs of the

coding sequence of NMDA3 correspond to 3' untranslated sequence of clone NMDA10 (i.e., nucleotides 4049-4279 in Sequence ID No. 1).

EXAMPLE 2

5 Preparation of full-length NMDAR1 subunit cDNA constructs

Portions of clones NMDA10, NMDA11, NMDA7 and NMDA3 were ligated together to construct full-length cDNAs encoding variants of the NMDA receptor NMDAR1 subunit. The full-length NMDAR1 subunit cDNAs were incorporated into vector pcDNA1 (Invitrogen, San Diego, CA) for use in expressing 10 the receptor subunits in mammalian host cells and for use in generating *in vitro* transcripts of the DNAs to be expressed in *Xenopus* oocytes.

Vector pcDNA1 is a pUC19-based plasmid that contains the following elements in the 5'-to-3' order: the cytomegalovirus (CMV) immediate early gene promoter/enhancer, the bacteriophage T7 RNA polymerase promoter, a polylinker, 15 the bacteriophage SP6 RNA polymerase promoter, SV40 RNA processing (i.e., splice donor/acceptor) signals, SV40 polyadenylation signal, and the ColE1 origin and supF suppressor tRNA to permit maintenance of the vector in *Escherichia coli* strains with the P3 episome. This vector thus contains all the regulatory elements required for expression of heterologous DNA in a mammalian host cell, wherein the heterologous 20 DNA has been incorporated into the vector at the polylinker. In addition, because the T7 and SP6 promoters are located on either side of the polylinker, this plasmid can be used for synthesis of *in vitro* transcripts of heterologous DNA that has been subcloned into the vector at the polylinker.

25 A. NMDAR1A

Full-length construct NMDAR1A was prepared by ligation of a 5' portion of NMDA11 (beginning 5' of the translation initiation codon and extending to the *Hind*III site in the middle of the clone) and a 3' portion of NMDA10 (beginning at 30 the *Hind*III site in the middle of the clone and extending 3' of the translation termination codon) as depicted in Figure 2. The two DNA fragments were joined in mammalian expression vector pcDNA1.

Initially, the strategy for generating the NMDAR1 construct involved a first step of separately subcloning the entire 4.0 kb *Eco*RI insert fragment of NMDA10 and the entire 4.0 kb *Sna*BI insert fragment of NMDA11 into pcDNA1; however, two attempts employing this cloning strategy were unsuccessful. It appeared 5 that there may have been selection against *E. coli* hosts retaining the complete insert fragments since the surviving recombinant *E. coli* that were analyzed contained incomplete insert cDNAs from which nucleotides had been deleted. Therefore, it was necessary to prepare the full-length NMDAR1A construct in several steps by subcloning and combining various fragments of NMDA10 and NMDA11 in pcDNA1 10 as follows (see Figure 3 for locations of restriction enzyme sites).

Clone NMDA10 was digested with *Bgl*II and *Eco*RI and the ~3.3 kb fragment containing nucleotides 1020-4298 of Sequence ID No. 1 was isolated and subcloned into *Bam*HI/*Eco*RI-digested pcDNA1. The resulting plasmid was digested with *Hind*III and *Nhe*I and the fragment containing nucleotides 2137-4298 of 15 Sequence ID No. 1 plus a portion of pcDNA1 was isolated.

Clone NMDA11 was digested with *Eco*RI and *Hind*III and the ~2.1 kb fragment containing nucleotides 1-2136 of Sequence ID No. 1 was isolated and subcloned into *Eco*RI/*Hind*III-digested modified pcDNA1 (modified by deletion of the *Hind*III site located 5' of the *Eco*RI site in the polylinker and addition of a *Hind*III 20 site into the polylinker at a position 3' of the *Eco*RI site). The resulting plasmid was digested with *Nhe*I and *Hind*III and the fragment containing nucleotides 1-2136 of Sequence ID No. 1 plus a portion of modified pcDNA1 was isolated. This *Nhe*I/*Hind*III fragment was then ligated to the *Hind*III/*Nhe*I fragment containing nucleotides 2137-4298 of Sequence ID No. 1 to generate the full-length construct 25 NMDAR1A (see Figure 2). The ligation mix was used to transform *E. coli* strain MC1061/P3. Because the *Nhe*I site in pcDNA1 occurs within the supF selection gene, only *E. coli* containing the correctly ligated, complete NMDAR1A plasmid (which has the complete, functional selection gene) were able to survive the selection process. This fragment subcloning strategy enabled selection of the desired correct 30 NMDAR1A-containing *E. coli* host cells, even though the total number of such recombinant host cells was small.

In summary, construct NMDAR1A contains 261 base pairs of 5' untranslated sequence from NMDAR11 (nucleotides 1-261 of Sequence ID No. 1) and a complete coding sequence (nucleotides 262-3078 of Sequence ID No. 1) for the 35 NMDAR1A variant of the NMDAR1 subunit as well as 1220 base pairs of 3'

untranslated sequence (nucleotides 3079-4298 of Sequence ID No. 1). The NMDAR1A-encoding sequence is operatively linked to the regulatory elements in pcDNA1 for expression in mammalian cells.

5 B. NMDAR1-Δ363

Full-length construct NMDAR1-Δ363 was prepared by ligation of a 5' portion of NMDA11 (beginning 5' of the translation initiation codon and extending to the *Hind*III site in the middle of the clone, i.e., nucleotides 1-2136 in Sequence ID No. 10 1) and a 3' portion of NMDA11 (beginning at the *Hind*III site in the middle of the clone and extending 3' of the translation termination codon, i.e., nucleotides 2137-2961 and 3325-4298 of Sequence ID No. 1). As described above, due to the difficulty in directly subcloning the entire 4.0 kb *Sna*BI NMDA11 insert into pcDNA1, it was necessary to generate the construct by ligating two fragments of the NMDA11 insert 15 into pcDNA1 as follows (see Figure 3 for locations of restriction enzyme sites).

To obtain the 5' NMDA11 fragment, clone NMDA11 was digested with *Eco*RI and *Hind*III and the ~2.2 kb fragment containing nucleotides 1-2136 of Sequence ID No. 1 was isolated and subcloned into *Eco*RI/*Hind*III-digested modified pcDNA1 (modified as described above). The resulting plasmid was digested with 20 *Nhe*I and *Hind*III and the fragment containing nucleotides 1-2136 of Sequence ID No. 1 plus a portion of modified pcDNA1 was isolated.

To obtain the 3' NMDA11 fragment, clone NMDA11 was digested with *Bgl*III and *Eco*RI and the 3.0 kb fragment containing nucleotides 1020-2961 and 3325-4298 of Sequence ID No. 1 was isolated and subcloned into *Bam*HI/*Eco*RI-digested pcDNA1. The resulting plasmid was digested with *Hind*III and *Nhe*I and the fragment containing nucleotides 2137-2961 and 3325-4298 of Sequence ID No. 1 plus a portion of pcDNA1 was isolated. This *Hind*III/*Nhe*I fragment was then ligated to the *Nhe*I/*Hind*III fragment containing nucleotides 1-2136 of Sequence ID No. 1 to generate NMDAR1-Δ363.

30 In summary, construct NMDAR1-Δ363 contains 261 base pairs of 5' untranslated sequence (nucleotides 1-261 of Sequence ID No. 1) and a complete coding sequence for the NMDAR1-Δ363 variant NMDAR1 subunit (nucleotides 262-2961 and 3325-3393 of Sequence ID No. 1) as well as 905 base pairs of 3' untranslated sequence (nucleotides 3394-4298 of Sequence ID No. 1). Thus, 35 NMDAR1-Δ363 differs from NMDAR1 in that it lacks 363 nucleotides (nucleotides

2962-3324 of Sequence ID No. 1) that comprise the last 117 nucleotides of the coding sequence and the first 246 nucleotides of the 3' untranslated sequence of NMDAR1. The NMDAR1-Δ363 subunit variant-encoding sequence is operatively linked to the regulatory elements in pcDNA1 for expression in mammalian cells.

5

C. NMDAR1-Δ1087

Full-length construct NMDAR1-Δ1087 was prepared by replacing the 3' end of the NMDAR1 variant-encoding insert of NMDAR1-Δ363 with a fragment 10 from the 3' end of clone NMDA3 (see Figure 2). Plasmid NMDAR1-Δ363 was partially digested with *Pst*I and completely digested with *Xba*I. There is a *Pst*I site ~112 nucleotides upstream of the location of the 363-nucleotide deletion in NMDAR1-Δ363 and an *Xba*I site in the polylinker located downstream of the 3' untranslated sequence of NMDAR1-Δ363 (see Figure 3). Thus, *Pst*I/*Xba*I digestion 15 of NMDAR1-Δ363 results in removal of a fragment containing nucleotides 2850-2961 and 3325-4298 of Sequence ID No. 1 from the vector. The larger fragment was isolated from the digest.

The insert of clone NMDA3 was cloned into the EcoRI restriction site(s) of pGEM (Promega, Madison, WI); and the resulting plasmid was digested 20 with *Pst*I and *Xba*I. The smaller fragment containing nucleotides 2850-2961 and 4049-4298 of Sequence ID No. 1 was isolated and ligated to the larger fragment from the *Pst*I/*Xba*I digest of NMDAR1-Δ363. The resulting construct was designated NMDAR1-Δ1087.

In summary, NMDAR1-Δ1087 contains 261 base pairs of 5' 25 untranslated sequence (nucleotides 1-261 in Sequence ID No. 1), the complete coding sequence for the NMDAR1-Δ1087 variant NMDAR1 subunit (nucleotides 262-2961 and 4049-4279 of Sequence ID No. 1) and 19 base pairs of 3' untranslated sequence (nucleotides 4280-4298 of Sequence ID No. 1). Thus, NMDAR1-Δ1087 differs from NMDAR1 in that it lacks 1087 nucleotides (nucleotides 2962-4048 of Sequence ID 30 No. 1) that comprise the last 117 nucleotides of the coding sequence and the first 970 nucleotides of the 3' untranslated sequence of NMDAR1. The NMDAR1-Δ1087 subunit variant-encoding sequence is operatively linked to the regulatory elements in pcDNA1 for expression in mammalian cells.

35 D. NMDAR1-I63-Δ204

Full-length construct NMDAR1-I63- Δ 204 was prepared by replacing a 1399-nucleotide fragment of construct NMDAR1A (i.e., nucleotides 738-2136 of Sequence ID No. 1) with the *Pvu*II-*Hind*III fragment of NMDA7 (i.e., nucleotides 5 738-831 of sequence ID No. 1, plus nucleotides 1-63 of Sequence ID No. 3 and nucleotides 832-984 and 1189-2136 of Sequence ID No. 1), as depicted in Figure 2. Because there are multiple *Pvu*II sites in the NMDAR1 construct, a several-step process was required for construction of NMDAR1-I63- Δ 204 as follows (see Figure 3 for the location of restriction enzyme sites).

10 The ~2.2-kb *Eco*RI-*Hind*III fragment isolated from construct NMDAR1A and containing nucleotides 1-2136 of Sequence ID No. 1 was ligated with modified pcDNA1 (modified as described in Example 2A) that had been digested with *Eco*RI and *Hind*III. The resulting plasmid was digested with *Avr*II and self-ligated to remove two *Pvu*II sites from a portion of the plasmid contributed by 15 pcDNA1. The plasmid was then partially digested with *Pvu*II and completely digested with *Hind*III. The digest was ligated with a 1258-nucleotide *Pvu*II-*Hind*III fragment isolated from clone NMDA7. The resulting plasmid, designated NMDAR1-I63- Δ 204-5', was digested with *Bam*HI and *Hind*III and the ~2-kb fragment containing nucleotides 1-831 of Sequence ID No. 1, plus nucleotides 1-63 of 20 Sequence ID No. 3 and nucleotides 832-984 and 1189-2136 of Sequence ID No. 1 was isolated and ligated to *Bam*HI/*Hind*III-digested NMDAR1 to generate NMDAR1-I63- Δ 204.

NMDAR1-I63- Δ 204 contains 261 base pairs of 5' untranslated sequence (nucleotides 1-261 in Sequence ID No. 1), the complete coding sequence for 25 the NMDAR1-I63- Δ 204 variant NMDAR1 subunit (nucleotides 262-831 of Sequence ID No. 1 plus nucleotides 1-63 of Sequence ID No. 3 and nucleotides 832-984 and 1189-3078 of Sequence ID No. 1) and 1220 base pairs of 3' untranslated sequence (nucleotides 3079-4298 of Sequence ID No. 1). Thus NMDAR1-I63- Δ 204 differs from NMDAR1 in that it contains 63 nucleotides that are not present in NMDAR1 30 (nucleotides 1-63 of Sequence ID No. 3) located between nt 831 and 832 of Sequence ID No. 1. Further, NMDAR1-I63- Δ 204 lacks 204 nucleotides that are present in NMDAR1 (nucleotides 985-1188 of Sequence ID No. 1). The NMDAR1-I63- Δ 204 subunit variant-encoding sequence is operatively linked to the regulatory elements in pcDNA1 for expression in mammalian cells.

E. NMDAR1-I63

Full-length construct NMDAR1-I63 can be described as NMDAR1 in
5 which a 173-bp fragment (nucleotides 738-910 of Sequence ID No. 1) is replaced
with the 236-bp *Pvu*II-*Sma*I fragment of NMDA7 (nucleotides 738-831 of Sequence
ID No. 1, plus nucleotides 1-63 of Sequence ID No. 3 and nucleotides 832-910 of
Sequence ID No. 1). Because there are multiple *Pvu*II sites in the NMDAR1
construct, a several-step process was required for construction of NMDAR1-I63 as
10 follows. Plasmid NMDAR1-I63-Δ204-5' was partially digested with *Sma*I and
completely digested with *Hind*III. The larger vector fragment was ligated with the
1226-bp *Sma*I/*Hind*III fragment isolated from NMDA11 (nucleotides 911-2136 of
Sequence ID No. 1). The resulting vector was digested with *Bam*HI and *Hind*III and
the ~2.2-kb fragment containing nucleotides 1-831 of Sequence ID No. 1, plus
15 nucleotides 1-63 of Sequence ID No. 3 and nucleotides 832-2136 of Sequence ID No.
1 was isolated and ligated to *Bam*HI/*Hind*III-digested NMDAR1 to generate
NMDAR1-I63.

NMDAR1-I63 contains 261 base pairs of 5' untranslated sequence
(nucleotides 1-261 in Sequence ID No. 1), the complete coding sequence for the
20 NMDAR1-I63 variant NMDAR1 subunit (nucleotides 262-831 of Sequence ID No. 1,
plus nucleotides 1-63 of Sequence ID No. 3 and nucleotides 832-3078 of Sequence ID
No. 1) and 1220 nucleotides of 3' untranslated sequence (nucleotides 3079-4298 of
Sequence ID No. 1). Thus, NMDAR1-I63 differs from NMDAR1 in that it contains
63 nucleotides that are not present in NMDAR1 (nucleotides 1-63 of Sequence ID No.
25 3), located between nucleotides 831 and 832 of Sequence ID No. 1. The
NMDAR1-I63 subunit variant-encoding sequence is operatively linked to the
regulatory elements in pcDNA1 for expression in mammalian cells.

F. NMDAR1-I63-Δ204-Δ363

30

Full-length construct NMDAR1-I63-Δ204-Δ363 was prepared by
replacing the 2861 nucleotide fragment from construct NMDAR1-I63-Δ204 (ie.
nucleotides 1438-4298 Sequence ID. No. 1) with the *Kpn*I-*Xba*I (polylinker site)
fragment of NMDAR1-Δ363 (ie. nucleotides 1438-2961 and 3325-4298 of Sequence
35 ID No. 1) as depicted in Figure 2. The NMDAR1-I63-Δ204 was completely digested

with *Xba*I then partially digested with *Kpn*I due to the presence of two additional *Kpn*I sites in the vector sequence. The resulting 5' NMDAR1-I63-Δ204 fragment, which includes the pcDNAI vector sequences, was ligated with the 3' *Kpn*I-*Xba*I fragment from NMDAR1-Δ363 to generate NMDAR1-I63-Δ204-Δ363.

5 In summary, construct NMDAR1-I63-Δ204-Δ363 contains 261 base pairs of 5' untranslated sequence (nucleotides 1-261 in Sequence ID No. 1), the complete coding sequence for the NMDAR1-I63-Δ204-Δ363 variant NMDAR1A subunit (nucleotides 262-831 of Sequence ID No. 1, plus nucleotides 1-63 of Sequence ID No. 3, plus nucleotides 832-984, 1189-2961 and 3325-3393 of Sequence 10 ID No. 1) as well as 905 base pairs of 3' untranslated sequence (nucleotides 3394-4298 of Sequence ID. No. 1). Thus, NMDAR1-I63-Δ204-Δ363 differs from NMDAR1A in that it contains 63 nucleotides that are not present in NMDAR1A (nucleotides 1-63 of Sequence ID No. 3) located between nucleotides 831 and 832 of Sequence ID No. 1. Further, NMDAR1-I63-Δ204-Δ363 lacks 204 nucleotides that 15 are present in NMDAR1A (nucleotides 985-1188 of Sequence ID No. 1) and 363 nucleotides that are present in NMDAR1A (nucleotides 2962-3324 of Sequence ID No. 1) that comprise the last 117 nucleotides of the coding sequence and the first 246 nucleotides of the 3' untranslated sequence of NMDAR1A. The NMDAR1-I63-Δ204-Δ363 subunit variant encoding sequence is operatively linked to the regulatory 20 elements in pcDNAI for expression in mammalian cells.

G. NMDAR1-I63-Δ204-Δ1087

Full-length construct NMDAR1-I63-Δ204-Δ1087 was prepared by 25 replacing the 2861 nucleotide fragment from construct NMDAR1-I63-Δ204 (ie, nucleotides 1438-4298 Sequence ID. N. 1) with the *Kpn*I-*Xba*I (polylinker site) fragment of NMDAR1-Δ1087 (ie, nucleotides 1438-2961 and 4049-4298 of Sequence ID No. 1) as depicted in Figure 2. The NMDAR1-I63-Δ204 was completely digested with *Xba*I then partially digested with *Kpn*I due to the presence of two additional 30 *Kpn*I sites in the vector sequence. The resulting 5' NMDAR1-I63-Δ204 fragment, which includes the pcDNAI vector sequences, was ligated with the 3' *Kpn*I-*Xba*I fragment from NMDAR1-Δ1087 to generate NMDAR1-I63-Δ204-Δ1087.

In summary, construct NMDAR1-I63-Δ204-Δ1087 contains 261 base pairs of 5' untranslated sequence (nucleotides 1-261 in Sequence ID No. 1), the complete coding sequence for the NMDAR1-I63-Δ204-Δ363 variant NMDAR1A subunit (nucleotides 262-831 of Sequence ID No. 1, plus nucleotides 1-63 of 5 Sequence ID No. 3, plus nucleotides 832-984, 1189-2961 and 4280-4298 of Sequence ID No. 1) as well as 19 base pairs of 3' untranslated sequence (nucleotides 4280-4298 of Sequence ID. No. 1). Thus, NMDAR1-I63-Δ204-Δ1087 differs from NMDAR1A in that it contains 63 nucleotides that are not present in NMDAR1A (nucleotides 1-63 of Sequence ID No. 3) located between nucleotides 831 and 832 of Sequence ID No. 10 1. Further, NMDAR1-I63-Δ204-Δ1087 lacks 204 nucleotides that are present in NMDAR1A (nucleotides 985-1188 of Sequence ID No. 1) and 1087 nucleotides that are present in NMDAR1A (nucleotides 2962-4048 of Sequence ID No. 1) that comprise the last 117 nucleotides of the coding sequence and the first 970 nucleotides 15 of the 3' untranslated sequence of NMDAR1A. The NMDAR1-I63-Δ204-Δ1087 subunit variant encoding sequence is operatively linked to the regulatory elements in pcDNAI for expression in mammalian cells.

H. Additional Constructs Containing Full-Length cDNAs Encoding Variants of the NMDAR1 Subunit

20

Additional full-length cDNAs encoding further possible NMDAR1 variants can be constructed using methods similar to those described in Examples 2A-G above. Specifically, the following constructs can be prepared by ligating portions of clones NMDA11, NMDA10, NMDA7 and NMDA3 as depicted in Figure 2:

25

	NMDAR1-Δ204	(Sequence ID No. 1J)
	NMDAR1-Δ204-Δ363	(Sequence ID No. 1K)
	NMDAR1-I63-Δ363	(Sequence ID No. 1M)
	NMDAR1-I63-Δ1087	(Sequence ID No. 1N)
30	NMDAR1-Δ204-Δ1087	(Sequence ID No. 1L)

The full-length cDNAs can also be incorporated into mammalian expression vectors such as pcDNA1, as described in Examples 2A-G.

Several methods can be employed to determine which NMDAR1
5 subunit variants are actually expressed in various human tissues. For example,
oligonucleotides specific for the nucleotide sequences located 5' and 3' of the
insertions and deletions of the NMDAR1 transcripts described herein can be used to
prime nucleic acid amplifications of RNA isolated from various tissues and/or cDNA
libraries prepared from various tissues. The presence or absence of amplification
10 products and the sizes of the products indicate which variants are expressed in the
tissues. The products can also be characterized more thoroughly by DNA sequence
analysis.

RNase protection assays can also be used to determine which variant
transcripts are expressed in various tissues. These assays are a sensitive method for
15 detecting and quantitating an RNA species in a complex mixture of total cellular
RNA. A portion of the NMDAR1 subunit variant DNA is labeled and hybridized
with cellular RNA. If complementary mRNA is present in the cellular RNA, a DNA-
RNA hybrid results. The RNA sample is then treated with RNase, which degrades
single-stranded RNA. Any RNA-DNA hybrids are protected from RNase degradation
20 and can be visualized by gel electrophoresis and autoradiography.

Further information on possible splice variants of the NMDAR1
primary transcript can be obtained by isolation of genomic clones containing
NMDAR1 subunit-encoding sequences (for example, by hybridization to the human
NMDAR1 subunit cDNAs disclosed herein) and subsequent characterization of the
25 resulting clones.

EXAMPLE 3

Isolation of DNA Encoding Human NMDA Receptor NMDAR2C Subunits

Degenerate oligonucleotides were synthesized based on two conserved
30 regions of rat NMDAR2A, NMDAR2B and NMDAR2C DNAs that encode the
putative first and fourth transmembrane domains. In rat NMDAR2A DNA, these
regions are encoded by nucleotides 1669-1692 (oligo SE74) and 2437-2465 (olig
SE75), respectively. [see Monyer *et al.* (1992) *Science* 256:1217-1221]. These

oligonucleotides were used to prime nucleic acid amplification of cDNAs prepared from RNA isolated from human hippocampus, cerebellum, and orbitofrontal tissue. Two products, a 795-bp and a 640-bp fragment, were detected when the reaction mixture was analyzed by gel electrophoresis and ethidium bromide staining. The 5 795-bp fragment amplified from the cerebellum cDNA was subcloned into PCR1000 (Invitrogen, San Diego, CA) and characterized by DNA sequence analysis, which revealed that it is ~86% similar to the rat NMDAR2A DNA sequence, ~78% similar to the rat NMDAR2B DNA sequence, and ~74% similar to the rat NMDAR2C DNA sequence. Thus, this plasmid was named pcrNMDAR2A.

10 The 795-bp insert from pcrNMDAR2A was used to screen 1 x 10⁶ recombinants of a human hippocampus cDNA library (prepared by using random primers to synthesize cDNAs from hippocampus tissue and selecting fragments >2.0 kb for insertion into λgt10 vectors) and a human cerebellum cDNA library (random-primed library size-selected for fragments >2.8 kb in λgt10). Hybridization was 15 performed in 5X SSPE, 5X Denhart's solution, 50% deionized formamide, 0.2% SDS, 200 μg/ml sonicated, denatured herring sperm DNA at 42°C. Washes were performed in 1X SSPE, 0.2% SDS at 55°C. The probe hybridized to 11 plaques from the hippocampus library and 8 plaques from the cerebellum library.

20 DNA sequence analysis and/or restriction enzyme mapping of 15 of the hybridizing plaques that were purified surprisingly revealed that they were more similar to rat NMDAR2C DNA than to rat NMDAR2A DNA. All of the clones were partial cDNAs (i.e., they lacked a translation initiation and/or termination codon) and were designated as NMDAR2C cDNAs. Comparison of the clones revealed that the human NMDAR2C subunit transcript is differentially processed.

25 Clones NMDA26, NMDA24, NMDA22 and NMDA21 (see Figure 4) represent four basic clones that were identified, all of which are believed to be splice variants. Clone NMDA26 (Sequence ID No. 5D) is used as a reference to which the other variants can be compared. Clone NMDA24 (Sequence ID No. 5C) contains a 24-bp sequence (see Sequence ID No. 7) that is not present in NMDA26. Clone 30 NMDA22 (Sequence ID No. 5B) lacks 15 bp that are present in NMDA26, and clone NMDA21 (Sequence ID No. 5A) lacks 51 bp that are present in NMDA26. Clones NMDA22 and NMDA24 both contain an 11-bp sequence (Sequence ID No. 9) that is not present in NMDA26 (between nucleotides 1116-1117 of Sequence ID No. 5). Introduction of this sequence into these clones (between nucleotides 1116-1117 of

Sequence ID No. 5) disrupts the reading frame of the transcript and introduces a premature translation termination (i.e., STOP) codon into the transcript.

Clones NMDA26 and NMDA27 (see Figure 4) are partial NMDAR2C cDNAs that contain 5' untranslated sequence, a translation initiation codon and some 5 of the coding sequence. Clone NMDA26 contains 188 base pairs of 5' untranslated sequence whereas clone NMDA27 contains ~1.1 kb of 5' untranslated sequence. The sequences of the 5' untranslated regions of these two clones are identical for the first 15 nucleotides proceeding 5' of the translation initiation codon. However, beginning with the 16th nucleotide 5' of the translation initiation codon, the sequences of the two 10 clones diverge (compare nucleotides 116-191 of Sequence ID No. 5 to nucleotides 1 - 74 of Sequence ID No. 12).

EXAMPLE 4

Preparation of Full-length NMDAR2C Subunit cDNA Constructs

15 Portions of the partial NMDAR2C clones can be ligated in a variety of ways to generate constructs encoding full-length NMDAR2C subunit variants. The 5' end of each NMDAR2C cDNA can be contributed by NMDA26, whereas the 3' ends of the constructs are contributed by various combinations of clones NMDA21, NMDA22, and NMDA24. Figure 5 depicts full-length NMDAR2C constructs and 20 indicates the portions of the different clones that contribute to each construct.

For example, full-length constructs can be prepared using methods such as those described in Example 2 for preparing NMDAR1 constructs. Thus, clone inserts are transferred into a vector (e.g., pcDNA1) for ease of manipulation and then desired portions of the cDNAs are isolated by restriction enzyme digestion of the 25 vectors. This can require several steps and/or partial digests if, for example, there are no unique restriction enzyme sites surrounding the desired portions of the cDNAs. The desired cDNA fragments are then ligated and incorporated into an expression plasmid such as pcDNA1 or pCMV-T7-2.

Plasmid pCMV-T7-2 (see Figure 6) is a pUC19-based vector that 30 contains a cytomegalovirus (CMV) promoter/enhancer, SV40 splice donor/splice acceptor sites located immediately downstream of the promoter, a T7 bacteriophage RNA polymerase promoter positioned downstream of the SV40 splice sites, an SV40 polyadenylation signal downstream of the T7 promoter, and a polylinker between the

T7 promoter and the polyadenylation signal. This vector thus contains all the regulatory elements required for expression of heterologous DNA in a mammalian host cell, wherein the heterologous DNA has been incorporated into the vector at the polylinker. In addition, because the T7 promoter is located just upstream of the 5 polylinker, this plasmid can be used for synthesis of *in vitro* transcripts of heterologous DNA that has been subcloned into the vector at the polylinker. Plasmid pCMV-T7-3, also depicted in Figure 6, is identical to pCMV-T7-2 except that the order of the restriction enzyme sites in the polylinker is reversed. This plasmid can also be used for heterologous expression of NMDAR subunit DNA.

10 Construct pcDNA1-26-NotI-24-5'UT contains 188 base pairs of 5' untranslated sequence (nucleotides 1-188 of Sequence ID No. 5), the complete coding sequence of the first variant of the human NMDAR2C subunit (nucleotides 189-3899 of Sequence ID No. 5) and ~440 base pairs of 3' untranslated sequence (nucleotides 3900-4340 of Sequence ID No. 5). The NMDAR2C cDNA is contained within the 15 polylinker of expression vector pcDNA1 for expression.

Construct pCMV-26-NotI-24 (Sequence ID No. 5) contains 49 base pairs of 5' untranslated sequence (nucleotides 140-188 of Sequence ID No. 5), the complete coding sequence of a first variant of the human NMDAR2C subunit (nucleotides 189-3899 of Sequence ID No. 5) and ~440 base pairs of 3' untranslated 20 sequence (nuceotides 3900-4340 of Sequence ID No. 5). The NMDAR2C cDNA is contained within the polylinker of expression vector pCMV-T7-2 for expression.

Construct pCMV-26-ScaI-24 (Sequence ID No. 5E) is identical to pCMV-26-NotI-24, except it contains 24-base pairs (Sequence ID No. 7) inserted between nucleotides 2350 and 2351 of Sequence ID No. 5.

25 Construct pCMV-26-ScaI-22 (Sequence ID No. 5F) is identical to pCMV-26-NotI-24, except that it lacks 15-base pairs (nucleotides 1960-1974 of Sequence ID No. 5).

Construct pCMV-26-ScaI-21-NotI-24 (Sequence ID No. 5G) is identical to pCMV-26-NotI-24, except that it lacks 51-base pairs (nucleotides 2351-30 2401 of Sequence ID No. 5).

Construct NMDAR2C-Δ15-I24 (Sequence ID No. 5H) is identical to pCMV-26-NotI-24, except that it lacks 15-base pairs (i.e., nucleotides 1960-1974 of Sequence ID No. 5) and includes a 24-base pair sequence (i.e., Sequence ID No. 7; inserted between nucleotides 2350 and 2351 of Sequence ID No. 5).

Construct NMDAR2C-Δ15-Δ51 (Sequence ID No. 5I) is identical to pCMV-26-NotI-24, except that it lacks 15-base pairs (i.e., nucleotides 1960-1974 of Sequence ID No. 5) and 51-base pairs (i.e., nucleotides 2351-2401 of Sequence ID No. 5).

5 Additional full-length NMDAR2C constructs can readily be prepared as described herein. For example, 5' untranslated sequence obtained from NMDA27 (instead of NMDA26) can be employed, and the 3' ends of the constructs can be contributed by various combinations of clones NMDA21, NMDA22, and NMDA24.

10 Several methods (e.g., nucleic acid amplification, RNase protection assays, etc.), as described in Example 2, can be employed to determine which 15 NMDAR2C subunit variants are actually expressed in various human tissues.

Human NMDAR2C has 83.5% GC nucleotide content between nucleotides 2957 and 3166. To potentially enhance NMDAR2D subunit expression, the GC content in this region can be reduced while maintaining the native amino acid 15 sequence. Synthetic DNAs can be made by oligonucleotide primer extension across this region. Four oligonucleotides, SE343 (Sequence ID No. 17), SE344 (Sequence ID No. 18), SE345 (Sequence ID No. 19), and SE346 (Sequence ID No. 20) were synthesized. These primers maintain the amino acid sequence of the human NMDAR2D receptor and some restriction sites, but lower the overall GC content of 20 this region to 53.4%. The criteria for the modification of bases were: 1) to not have more than 4 guanine nucleotides in a row if at all possible, 2) to maintain the restriction cutting sites for *NotI* (nucleotides 2962 - 2969 of Sequence ID No. 5), *AvaII* (nucleotides 3069 - 3073 Sequence ID No. 5), and *AatII* (nucleotides 3156 - 3161 of Sequence ID No. 5), 3) to reduce the secondary structure of the 25 oligonucleotides as much as possible, 4) to not introduce any additional *NotI*, *AvaII* or *AatII* restriction sites into the sequence and 5) to have the basepair overlap between oligonucleotide pairs, {SE343 and SE344} or {SE345 and SE346} have a proposed melting temperature between 62-66°C. The oligonucleotide pair SE343 and SE344 have complementary sequence from nucleotides 51 - 71 of Sequence ID Nos. 17 and 30 18. The oligonucleotide pair SE345 and SE346 have complementary sequence from nucleotides 42 - 61 of Sequence ID No. 19 and nucleotides 43 - 62 of Sequence ID No. 20, respectively.

The primer pairs, {SE343 and SE344} and {SE345 and SE346}, are combined in a standard PCR reaction mixture, which contains 50 pmoles of each 35 oligonucleotide, and are amplified according to the following PCR protocol:

Annealing temperature of 55°C for 1 min, extension temperature of 72°C for 2 min and melting temperature, 96°C for 30 seconds for 30 cycles.

5

The resulting 121 bp PCR product from the primer pair SE343-SE344 is digested with *NotI* and *AvaI*, and the resulting 103 bp PCR product from the primer pair SE345-SE346 is digested with *AvaI* and *AatII*. These fragments are ligated into pCMV-NMDAR2C-26-*NotI*-24, which has been partially digested with both *NotI* and *AatII* due to the presence of additional *NotI* and/or *AatII* restriction sites in the vector sequence, to form pCMV-26-*NotI*-24-GCMOD. This construct, pCMV-26-*NotI*-24-GCMOD, contains nucleotides 140-2965 of Sequence ID No. 5, followed by the 195 nucleotides set forth in Sequence ID No. 21, and then nucleotides 3161 to 4340 of Sequence ID. No. 5.

15

EXAMPLE 5

Isolation of DNA Encoding Human NMDA Receptor NMDAR2A Subunits

Two human cDNA libraries were prepared using different oligonucleotides (random and specific primers) to prime cDNA synthesis from RNA isolated from cerebellum tissue. The specific primer used for first-strand synthesis was SE162, nucleotides 904 to 929 of Sequence ID No. 10. cDNAs synthesized by random priming that ranged in size from 1.0-2.8 kb, and cDNAs synthesized by specific priming that ranged in size from 0.6-1.1 kb, were isolated and inserted into the λgt10 phage vector to generate the two libraries.

25 The random-primed library (3×10^6 recombinants) was screened for hybridization to the 795-base pair insert from pcrNMDAR2A (see Example 3) in 5X SSPE, 5X Denhart's solution, 50% deionized formamide, 0.2% SDS, 200 µg/ml sonicated, denatured herring sperm DNA at 42°C. Washes were performed in 1X SSPE, 0.2% SDS at 55°C. The probe hybridized to 11 plaques.

30 The specifically-primed library (6×10^5 recombinants) was screened for hybridization to oligonucleotide SE177 (nucleotides 859 to 884 of Sequence ID No. 10) in 6X SSPE, 5X Denhart's solution, 10% deionized formamide, 0.2% SDS,

200 µg/ml sonicated, denatured herring sperm DNA at 42°C. Washes were performed in 1X SSPE, 0.2% SDS at 50°C. The probe hybridized to 2 plaques.

Nine of the hybridizing plaques were purified and the inserts were characterized by restriction enzyme mapping and DNA sequence analysis. All clones 5 contained partial cDNAs. Two of the clones, NMDA53 and NMDA54, contain the translation initiation codon and 320 base pairs and 88 base pairs, respectively, of 5' untranslated sequence. The sequences of four other clones, NMDA47, NMDA49, NMDAR50 and NMDA51, along with those of NMDA53 and NMDA54, overlap to comprise ~70% of the human NMDAR2A subunit coding sequence (see nucleotides 10 - 3084 of Sequence ID No. 10).

To obtain clones containing the remaining ~1300 base pairs of 3' sequence needed to complete the NMDAR2A coding sequence, 6.6×10^6 recombinants of an additional human cDNA library (an amplified randomly primed cerebellum cDNA library with inserts ranging from 1.0 - 2.8 kb in length) were 15 screened for hybridization to an oligonucleotide corresponding to the 3' end of clone NMDA51 (oligo SE171; nucleotide 3454 to 3479 of Sequence ID No. 10) using the same conditions as used for screening the specifically primed cerebellum cDNA library as described above. Four hybridizing plaques were purified and the inserts were characterized by DNA sequence analysis to determine if they contain the 3' end 20 of the coding sequence and a translation termination codon. Two of the clones (NMDA57 and NMDA58, which were determined to be identical), contain a translation termination codon, as determined by DNA sequence analysis. Phage lysate containing clone NMDA57 were deposited under the provisions of the Budapest Treaty with the American Type Culture Collection (ATCC) on April 13, 25 1993, and assigned Accession No. 75442.

EXAMPLE 6

Preparation of Full-length NMDAR2A Subunit cDNA Constructs

Two separate constructs encoding a full-length NMDAR2A subunit 30 (pCMV-hNMDAR2A-1(53) and pCMV-hNMDAR2A-2(54) were prepared by ligating portions of the following partial NMDAR2A clones: NMDAR47, NMDAR50, NMDAR58 and either NMDAR53 or NMDAR54 (NMDAR53 and NMDAR54 differ only in the amount of 5' untranslated sequence contained in the

clones. The inserts of clones NMDA47, NMDA50 and NMDA58 were isolated as *Eco*RI fragments and ligated with *Eco*RI-digested pCMV-T7-2 to create pNMDA47, pNMDA50 and pNMDA58, respectively. The inserts of clones NMDA53 and NMDA54 were isolated as *Xba*I fragments and ligated with *Sac*I-digested pCMV-T7-2 to create pNMDA53 and pNMDA54, respectively.

pNMDA47 was digested with *Sca*I and *Nsi*I to liberate an ~3,350-bp fragment containing a 3' portion of the β-lactamase gene, which encodes a protein which imparts ampicillin-resistance, and nucleotides 824-2415 of Sequence ID No. 10. This fragment was ligated with a ~2890-bp *Nsi*I/*Sca*I fragment of pNMDA50 (containing a 5' portion of the β-lactamase gene and nucleotides 2416-3346 of Sequence ID No. 10) to generate pNMDA47+50.

The portion of pNMDA58 that encodes the 3' end of NMDAR2A contains two *Msc*I sites. Because the 3' *Msc*I site is cleaved in preference to the 5' *Msc*I site, partial digestion of pNMDA58 was not an option. Thus, pNMDA58 was digested with *Sca*I/*Msc*I, and the ~2020-bp fragment containing a 5' portion of the β-lactamase gene and a 3' portion of the insert (nucleotides 4751-4808 of Sequence ID No. 10) was isolated. This fragment was ligated to a ~4150-bp *Sca*I/*Msc*I fragment of pNMDA47+50 (containing a 3' portion of the β-lactamase gene and nucleotides 824-3212 of Sequence ID No. 10) to generate pNMDA47+50+3'END58. This plasmid contained a complete β-lactamase gene and nucleotides 824-3214 and 4751-4808 of Sequence ID No. 10. To add nucleotides 343-4750 of Sequence ID No. 10 to pNMDA47+50+3'END58, pNMDA58 was digested with *Msc*I, and the isolated 1537-bp fragment consisting of nucleotides 3213-4750 of Sequence ID No. 10 was ligated to *Msc*I-digested pNMDA47+50+3'END58. The resulting plasmid, pNMDA47+50+58, contained nucleotides 824-4808 of Sequence ID No. 10.

To generate two constructs containing identical NMDAR2A coding sequences but differing amounts of 5' untranslated sequence, pNMDA53 and pNMDA54 were digested with *Sca*I/*Eco*RI to liberate fragments containing a 3' portion of the β-lactamase gene and nucleotides 1-854 and 225-854 of Sequence ID No. 10, respectively. pNMDA47+50+58 was digested with *Sca*I/*Eco*RI (partial) and the 3954-bp fragment containing a 5' portion of the β-lactamase gene and nucleotides 855-4808 of Sequence ID No. 10 was separately ligated with the *Sca*I/*Eco*RI fragments of pNMDA53 and pNMDA54 to generate pCMV-hNMDAR2A-1(53) and pCMV-hNMDAR2A-2(54), respectively. These two constructs are identical except for the amount of 5' untranslated sequence contained in each. Both contain a full-

length NMDAR2A-encoding sequence (nucleotides 311-4705 of Sequence ID No. 10) and 103 nucleotides of 3' untranslated sequence (nucleotides 4706-4808 of Sequence ID No. 10). pCMV-hNMDAR2A-1(53) contains 310 nucleotides of 5' untranslated sequence (nucleotides 1-310 of Sequence ID No. 10), whereas pCMV-hNMDAR2A-5 2(54) contains 87 nt of 5' untranslated sequence (nucleotides 224-310 of Sequence ID No. 10). The NMDAR2A cDNA is operatively linked to the regulator elements of pCMV-T7-2 for expression in mammalian host cells.

There is no unique restriction site 3' of the NMDAR2A-specific DNA in pCMV-hNMDAR2A-1(53) that can be used to linearize the plasmid in order to 10 prepare *in vitro* transcripts for injection into *Xenopus* oocytes. To make a construct that has a unique 3' restriction site (pCMV-hNMDAR2A-3(53)), essentially the entire NMDAR2A-specific DNA of pCMV-hNMDAR2A-1(53) was transferred into vector pCMV-T7-3 as follows. pCMV-NMDAR2A-1(53) was digested with *NotI* and the ~4.4-kb fragment was isolated and ligated with *NotI*-digested pCMV-T7-3 to generate 15 pCMV-hNMDAR2A-3(53).

EXAMPLE 7

Isolation of DNA Encoding Human NMDA Receptor NMDAR2B Subunits

A human fetal brain λZAP cDNA library (1×10^6 recombinants; 20 Stratagene, La Jolla, CA) was screened for hybridization to a DNA fragment containing the entire rat NMDAR2B subunit coding sequence (see Monyer et al. (1992) Science 256:1217-1221). Hybridization was conducted in 50% deionized formamide, 5X Denhart's solution, 5X SSPE, 200 µg/ml sonicated, denatured herring sperm DNA and 0.2% SDS at 42°C. Washes were performed in 0.5X SSPE, 0.2% 25 SDS at 65°C. One of the hybridizing clones excised from the human fetal brain library, NMDA81, containing a 5,435 bp insert and translation initiation and termination codons, encodes a full-length NMDAR2B subunit. This excised plasmid, which is in the pBluescript vector, was called pBS-hNMDAR2B.

NMDA81 was digested with *EcoRI/EcoRV* and the ~5.5-kbp fragment 30 was isolated and ligated to *EcoRI/EcoRV*-digested pCMV-T7-3. The resulting construct, pCMVPL3-hNMDAR2B, contains the NMDAR2B coding sequence (nucleotides 210-4664 of Sequence ID No. 13), as well as 209 nucleotides of 5' untranslated sequence (nucleotides 1-209 of Sequence ID No. 13) and 339 nucleotides

of 3' untranslated sequence (nucleotides 4665-5003 of Sequence ID No. 13). The NMDAR2B-encoding DNA in this construct is operatively linked to regulatory elements in pCMV-T7-3 for expression in mammalian host cells.

5

EXAMPLE 8

Isolation of DNA Encoding Human NMDA Receptor NMDAR2D subunits

A human fetal brain cDNA library (1×10^6 recombinants; Stratagene, La Jolla, CA) was screened by subtraction screening methods for DNA encoding a human NMDAR2D receptor subunit. In this method, plaques were selected on the basis of weak or no hybridization to DNAs encoding human NMDAR2A, NMDAR2B and NMDAR2C subunits.

Initially, the library was screened for hybridization to pcrNMDAR2A (see Example 3) under low-stringency conditions (30% deionized formamide, 5X Denhart's solution, 5X SSPE, 200 ng/ml sonicated herring sperm DNA, 0.2% SDS at 42°C). Washes were also performed using low-stringency conditions (2X SSPE, 0.2% SDS, 50°C). The filters were stripped, then screened for hybridization to the pcrNMDAR2A fragment and to an ~1200 bp PstI fragment of DNA encoding a human NMDAR2B subunit (see Example 7) and an ~950 bp AccI fragment of DNA encoding a human NMDAR2C subunit (see Example 3). These fragments contain DNA encoding all of the putative transmembrane domains of the subunits. Hybridization was performed under high-stringency conditions (50% deionized formamide, 5X Denhart's solution, 5X SSPE, 200 ng/ml sonicated herring sperm DNA, 0.2% SDS at 42°C) as were washes (0.1X SSPE, 0.1% SDS, 65°C).

Eighteen of the plaques that hybridized weakly to pcrNMDAR2A in the initial low stringency screening of the library hybridized only weakly or not at all to portions of DNA encoding human NMDAR2A, NMDAR2B and NMDAR2C subunits in the high stringency screening. The plaques were purified, and the insert fragments were characterized by DNA sequence analysis. One of the inserts, NMDA96, corresponds to the 3' half of the human NMDAR2D subunit gene coding sequence. The sequence of this clone is provided in Sequence ID No. 15.

To obtain clones containing the remaining ~2000 bp of 5' sequence needed to complete the NMDAR2D subunit coding sequence, the human fetal brain cDNA library was screened for hybridization to an ~831 bp *Sma*I fragment of the clone containing the 3' half of the NMDAR2D coding sequence under high stringency
5 hybridization and washing with 0.5X SSPE, 0.2% SDS at 65°C. Nine hybridizing plaques were purified and analyzed by DNA sequencing, which revealed that none of the plaques contain DNA encoding a translation initiation codon and extending 3' to at least the 5' end of the clone containing the 3' half of the NMDAR2D coding sequence.

A human cDNA library was prepared using a specific oligonucleotide,
10 SE296, to prime cDNA synthesis from RNA isolated from human fetal brain. The specific primer used for first-strand synthesis was SE296 (nucleotides 2920-2949 of Sequence ID No. 15). cDNAs synthesized by specific priming that were greater than 2.2 kb in size were isolated and inserted into the λZAPII phage vector to generate the library.

15 The specifically primed library (1×10^6 recombinants) was screened for hybridization to the 831 bp *Sma*I fragment from NMDAR2D (nucleotides 2435-3265 of Sequence ID No. 15) in 5X SSPE, 5X Denhart's solution, 50% deionized formamide, 0.2% SDS, 200 µg/ml sonicated, denatured herring sperm DNA at 42°C. Washes were performed in 0.1X SSPE, 0.2% SDS at 65°C. One probe hybridized to
20 11 plaques.

Eleven of the hybridizing plaques were purified, and the inserts characterized by restriction enzyme mapping and DNA sequence analysis. Six of the clones (NMDA111, NMDA112, NMDA115, NMDA116, NMDA119 and NMDA121) contain the translation initiation codon and varying amounts of 5'
25 untranslated sequence.

The sequences of these clones overlap with NMDA96 to constitute 100% of the human NMDAR2D subunit coding sequence (see nucleotides 485-4495 of Sequence ID No. 15).

30 The full-length hNMDAR2D construct was prepared using NMDA115 and NMDA96 cDNAs. NMDA115 and NMDA96 cDNAs are already in the pBlueScript vector, however the NMDA115 cDNA is in the sense orientation from the T7 promoter, while the NMDA96 cDNA is in the antisense orientation. For ease of subcloning the full-length construct, the NMDA96 cDNA was cloned into the
sense orientation by digesting NMDA96 with *Eco*RI and screening the resulting
35 clones for orientation (NMDA96-T7). Within the complete human NMDAR2D

sequence, there is a unique *Hind*III at nucleotides 2804 that was used to clone NMDA115 together with NMDA96. However, there is an additional *Hind*III site in the pBS polylinker at the 5' end of the NMDA115 cDNA. Therefore NMDA115 was fully digested with *Spe*I, a 3' polylinker site, and partially digested with *Hind*III. The resulting ~5.6 kb *Spe*I-*Hind*III fragment from pNMDA115 (pBS vector plus nucleotides 397-2804 of Sequence ID No. 15)) was ligated with the 1.7 kb *Hind*III-*Spe*I fragment (nucleotides 2805-4651 of Sequence ID No. 15) from NMDA96-T7 to form pBS-hNMDAR2D. *In vitro* transcripts were prepared for co-injection into *Xenopus* oocytes to test for alteration of NMDAR1A currents.

10 The complete NMDAR2D insert is then transferred into the pMMTV-T7+ mammalian expression vector as a ~4.7 kb *Eco*RV/*Spe*I fragment. The *Eco*RV and *Spe*I restriction sites are in the multiple cloning region of the pBluscript vector.

15 In summary, construct NMDAR2D contains 88 base pairs of 5' untranslated sequence (nucleotides 397-484 in Sequence ID No. 15), the complete coding sequence for the NMDAR2D subunit (nucleotides 484-4495 of Sequence ID No. 15) as well as 200 base pairs of 3' untranslated sequence (nucleotides 4496-4695 of Sequence ID No. 15). The NMDAR2D subunit encoding sequence is operatively linked to the regulatory elements in pMMTV-T7 for expression in mammalian cells.

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EXAMPLE 9

Expression of Recombinant Human NMDA Receptor Subunits on Oocytes

25 *Xenopus* oocytes were injected with *in vitro* transcripts prepared from constructs containing DNA encoding human NMDA receptor NMDAR1 and NMDAR2 subunits. Electrophysiological measurements of the oocyte transmembrane currents were made using the two-electrode voltage clamp technique (see e.g., Stuhmer (1992) *Meth. Enzymol.* 207:319-339).

A. Preparation of *In Vitro* Transcripts

30 Recombinant capped transcripts of NMDA receptor subunit cDNAs contained in constructs NMDAR1A, NMDAR1-I63, NMDAR1-I63-Δ204, NMDAR1-Δ1087, NMDAR1-Δ363, and pCMV-26-NotI-24 were synthesized from linearized plasmids using the mCAP RNA Capping Kit (Cat. #200350, Stratagene,

Inc., La Jolla, CA). For experiments in which NMDAR2A or NMDAR2B and NMDAR1 or NMDAR1-I63 transcripts were co-injected into *Xenopus* oocytes, the transcripts were synthesized from linearized constructs NMDAR1A, NMDAR1-I63, pCMV-hNMDAR2A-3(53), pCMV-26-*NotI*-24 and pBS-hNMDAR2B using 5 mMessage mMachine (Ambion, catalog #1344, Austin, TX). The mass of each synthesized transcript was determined by UV absorbance and the integrity of each transcript was determined by electrophoresis through an agarose gel.

B. Electrophysiology
10 *Xenopus* oocytes were injected with 12.5-50 ng of one or more NMDA receptor subunit transcripts per oocyte. The preparation and injection of oocytes were carried out as described by Dascal [(1987) *Crit. Rev. Biochem.* 22:317-387]. Two-to-six days following mRNA injection, the oocytes were examined using the two-
15 electrode voltage clamp technique. The cells were bathed in Ringer's solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl₂, 10 mM HEPES, pH 7.3), and the membrane potential was clamped at -80 to -100 mV. Drugs were applied by pipetting 6.0 µl aliquots of drug-containing solution directly into the bath, or by using gravity-feed into a Warner Instruments chamber (volume = 110 µl) at a flow rate of 8 ml/min. The
20 data were sampled at 2-5 Hz with a Labmaster data acquisition board in a PC-386 using AXOTAPE version 1.2 (Axon Instruments, Foster City, CA) software. The data were exported to a laser printer or plotted using SigmaPlot version 5.0.

NMDA agonists, i.e., 10-30 µM glycine (gly) and 10-100 µM glutamate (glu) or 100-1000 µM NMDA, were applied to the bath. If a current
25 response was observed, the agonists were washed from the bath and 0.1-1.0 mM MgCl₂ or 1 µM MK801 (Research Biochemicals, Inc., Natick, MA) (NMDA receptor antagonists) were applied before a second agonist application in order to determine whether the current was blocked by antagonists. Alternatively, MgCl₂ or MK-801 were applied during agonist-induced current flow. The results of multiple
30 recordings are summarized in Table 1.

Table 1

Electrophysiological Analysis of Oocytes Injected with
NMDA Receptor Subunit Transcripts

Transcript (ng injected)	No. Oocytes Responding	Agonists	Peak Current Amplitude
NMDAR1A (12.5)	6 of 8 ^a	10 μM gly + 10 μM glu	3-40 nA*
NMDAR1A (12.5)	2 of 2 ^a	10 μM gly + 100 μM NMDA	3-8 nA
NMDAR1A (12.5)	0 of 9 ^a	10 μM gly + 10 μM glu	
NMDAR1A (50)	0 of 1 ^a	20 μM gly + 20 μM glu	
NMDAR1A (40)	4 of 10	10 μM gly + 10 μM glu	21.3 ± 20.9 nA*
NMDAR1A (40)	1 of 5	10 μM gly + 100 μM NMDA	24 nA*
NMDAR1A (40)	1 of 1	10 μM gly + 100 μM NMDA	15.4 nA
NMDAR1A (30)	4 of 9	10 μM gly + 50 μM glu	10.6 ± 11.7 nA*
NMDAR1A (30)	0 of 8	10-20 μM gly + 10-100 μM glu	
NMDAR1A (30)	1 of 4	20 μM gly + 100 μM NMDA	10.5 nA

Transcript (ng injected)	No. Oocytes Responding	Agonists	Peak Current Amplitude
NMDARI α (25-50)	3 of 3	30 μ M gly + 100 μ M glu	3-10 nA
NMDARI-163 (12.5)	1 of 5 ^a	10 μ M gly + 10 μ M glu	~30 nA*
NMDARI-163 (50)	0 of 4 ^a	10 μ M gly + 10 μ M glu	
NMDARI-163 (40)	4 of 5	10 μ M gly + 10 μ M glu	13.4 ± 7.1 nA ⁺
NMDARI-163 (40)	3 of 3	10 μ M gly + 20 μ M glu	17.4 ± 3.7 nA*
NMDARI-163 (40)	1 of 1	10 μ M gly + 100 μ M glu	28 nA
NMDARI-163 (40)	1 of 1	10 μ M gly + 10 μ M NMDA	1.4 nA*
NMDARI-163 (25-50)	3 of 3	10 μ M gly + 100 μ M glu	3-5 nA
NMDARI-163 (40)	7 of 10	10 μ M gly + 100 μ M NMDA	8.1 ± 3.0 nA*
NMDARI-163 (40)	1 of 2	10 μ M gly + 1000 μ M NMDA	16.4 nA ⁺
NMDARI-163-Δ204 (12.5)	0 of 8 ^a	10 μ M gly + 10 μ M glu	
NMDARI-163-Δ204 (50)	1 of 5 ^a	20 μ M gly + 20 μ M glu	~50 nA
NMDARI-Δ1087 (50)	3 of 13	10 μ M gly + 10 μ M glu	4-11 nA*

Transcript (ng injected)	No. Oocytes Responding	Agonists	Peak Current Amplitude
NMDAR1A (39) + pCMV-26-NotI-24 (39)	1 of 5	10 μ M gly + 50 μ M glu	10 nA
NMDAR1A (30) + pCMV-26-NotI-24 (30)	0 of 7	10 μ M gly + 20 μ M glu	
NMDAR1A (32) + pcDNA1-26-NotI-24-5UT (50)	4 of 5	10 μ M gly + 10 μ M glu	15.8 \pm 2.6 nA
NMDAR1A (25-50) + pCMV-hNMDAR2A-3(53) (25-50)	16 of 29	30 μ M gly + 100 μ M glu	40 nA - 3.4 μ A
NMDAR1-163 (25-50) + pCMV-hNMDAR2A-3(53) (25-50)	6 of 11	10 μ M gly + 100 μ M glu	10 - 100 nA
NMDAR1A (25) + pBS-hNMDAR2B (25)	4 of 5	30 μ M gly + 30 μ M glu	>100 nA
NMDAR1A (50) + pCMV-hNMDAR2A-3 (50) +	15 of 22	100 μ M NMDA + 30 μ M gly -or-	137.7 nA

Transcript (ng injected)	No. Oocytes Responding	Agonists	Peak Current Amplitude
pCMV-26-NotI-24 (50)		100 μ M NMDA + 100 μ M gly	1340.1 nA

- a Oocytes were unhealthy (i.e., the holding current was large)
- * The agonist-induced currents in at least 1 cell were blocked by 100 μ M MgCl₂.
- + The agonist-induced currents in at least 1 cell were blocked by 1.0 μ M MK801.

Analysis of the results shown in Table 1 indicates that, in general, the NMDA agonist-induced currents were blocked by either MgCl₂ or MK801.

Oocytes injected with transcripts (12.5 to 65 ng) of the NMDAR-1 subunit-encoding inserts of constructs NMDAR1A, NMDAR1-I63 or NMDAR1-Δ363 were further analyzed to evaluate human NMDA receptor sensitivity to glutamate and NMDA. The two-electrode voltage clamp methods described above were used to measure current in the cells.

To determine glutamate and NMDA sensitivity of the recombinant human NMDA receptors, various concentrations of glutamate (0.1 - 100 μM) or NMDA (3-1000 μM) were applied to the bath (in the presence of 10-30 μM glycine) and the current response was recorded. The bath was flushed between agonist applications. Intermediate test applications of 10 μM glycine plus 10 μM glutamate were included in the experiments to monitor the receptors for run-down (i.e., inactivation of receptors that have been repeatedly activated during prolonged electrophysiological recording). The data were used to generate dose-response curves from which EC₅₀ values for the two agonists were calculated. Glycine sensitivity was determined in the same manner except that various concentrations (0.1-100 μM) of glycine were co-applied with 100 μM NMDA.

The EC₅₀ values determined for glutamate stimulation of NMDA receptors expressed in oocytes injected with NMDAR1A, NMDAR1-I63 or NMDAR1-Δ363 transcripts were 0.4, 0.6 and 0.5 μM, respectively. The EC₅₀ values determined for NMDA stimulation of NMDA receptors expressed in oocytes injected with NMDAR1A, NMDAR1-I63 or NMDAR1-Δ363 transcripts were 6.3, 10.9 and 11.9 μM, respectively.

There was a marked potentiation of the current magnitude in response to glutamate and glycine in oocytes co-injected with *in vitro* transcripts of pCMV-hNMDAR2A-3(53) and NMDAR1A or NMDAR1-I63 compared to the currents recorded in oocytes injected with transcripts of either NMDAR1A or NMDAR1-I63 alone. Similarly, there was a marked potentiation of the current magnitude in response to glutamate and glycine in oocytes co-injected with *in vitro* transcripts of NMDAR1A and pBS-hNMDAR2B compared to the currents recorded in oocytes injected with only the NMDAR1A transcript.

To investigate the pharmacological properties of human NMDA receptors generated by coexpression of the human NMDAR1A, NMDAR2A and NMDAR2C subunits, oocytes were co-injected with 50 ng each of *in vitro* transcripts

prepared from the NMDAR1A, pCMV-hNMDAR2A-3, and pCMV-26-NotI-24 (NMDAR2C) constructs. The sensitivity of the recombinant heteromeric receptors to glycine and NMDA was determined as described above. The EC₅₀ for glycine activation of inward currents in these recombinant oocytes was calculated from the dose-response curve to be $0.87 \pm 0.24 \mu\text{M}$ (mean \pm S.D. of 4 oocytes), which was significantly different than the EC₅₀ calculated for glycine sensitivity of oocytes injected with 50 ng each of *in vitro* transcripts of NMDAR1A and pCMV-hNMDAR2A-3 alone ($1.9 \pm 0.26 \mu\text{M}$; $p = 0.0002$, one-tailed t-test). The sensitivity to NMDA also increased when human NMDAR2C was co-expressed with human NMDAR1A and NMDAR2A subunits. The EC₅₀ for NMDA was shifted from $30.2 \pm 9.4 \mu\text{M}$ for oocytes co-injected with 50 ng each of *in vitro* transcripts of NMDAR1A and pCMV-hNMDAR2A-3 to $11.9 \pm 5.2 \mu\text{M}$ for oocytes co-injected with 50 ng each of *in vitro* transcripts of NMDAR1A, pCMV-hNMDAR2A-3 and pCMV-26-NotI-24 (mean \pm S.D. of 4 oocytes).

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EXAMPLE 10

Recombinant Expression of Human NMDA Receptor Subunits in Mammalian Cells

Mammalian cells, such as human embryonic kidney (HEK293) cells can be transiently and/or stably transfected with DNA encoding human NMDA receptor subunits (e.g., DNA encoding an NMDAR1 subunit or DNA encoding an NMDAR1 subunit and DNA encoding an NMDAR2 subunit such as pCMV-26-NotI-24, pCMV-hNMDAR2A-3(53) or pCMVPL3-hNMDAR2B). Transfectants are analyzed for expression of NMDA receptors using various assays, e.g., northern blot hybridization, electrophysiological recording of cell currents, Ca²⁺-sensitive fluorescent indicator-based assays and [³H]-MK801 binding assays.

A. Transient Transfection of HEK Cells

Two transient transfections were performed. In one transfection, HEK 293 cells were transiently transfected with DNA encoding an NMDAR1 (construct NMDAR1A) subunit. In another transfection, HEK 293 cells were transiently co-transfected with DNA encoding NMDAR1 (construct NMDAR1A) and NMDAR2C (pCMV-26-NotI-24) subunits. In both transfections, $\sim 2 \times 10^6$ HEK cells were

transiently transfected with 19 µg of the indicated plasmid(s) according to standard CaPO₄ transfection procedures [Wigler *et al.* (1979) *Proc. Natl. Acad. Sci. USA* 76:1373-1376]. In addition, 1 µg of plasmid pCMVβgal (Clontech Laboratories, Palo Alto, CA), which contains the *Escherichia coli* β-galactosidase gene fused to the
5 CMV promoter, were co-transfected as a reporter gene for monitoring the efficiency of transfection. The transfectants were analyzed for β-galactosidase expression by direct staining of the product of a reaction involving β-galactosidase and the X-gal substrate [Jones (1986) *EMBO* 5:3133-3142]. Transfectants can also be analyzed for β-galactosidase expression by measurement of β-galactosidase activity [Miller (1972)
10 in *Experiments in Molecular Genetics*, pp.352-355, Cold Spring Harbor Press].

The efficiency of these transfections of HEK cells was typical of standard efficiencies (i.e., ~50%).

B. Stable Transfection of Mammalian Cells

15 Mammalian cells, such as HEK 293 cells, can be stably transfected using the calcium phosphate transfection procedure [*Current Protocols in Molecular Biology*, Vol. 1, Wiley Inter-Science, Supplement 14, Unit 9.1.1-9.1.9 (1990)]. Ten-cm plates, each containing 1-2 x 10⁶ cells, are transfected with 10 ml of
20 DNA/calcium phosphate precipitate in media containing approximately 19 µg of NMDA receptor subunit-encoding DNA and 1 µg of DNA encoding a selectable marker, for example, neomycin-resistance gene (i.e., pSV2neo). After ~14 days of growth in media containing typically 1 µg/ml G418, colonies form and are individually isolated using cloning cylinders. The isolates are then subjected to
25 limiting dilution and screened to identify those that express NMDA receptors using, for example, methods described below.

C. Analysis of Transfectants

30 1. Northern Blot Hybridization Analysis

Total RNA was isolated from ~1 x 10⁷ HEK cells co-transfected with NMDAR1 and pCMV-26-NotI-24, and 5-10 µg of RNA was used for northern hybridization analysis. Fragments from human neuronal NMDAR subunit-encoding
35 plasmids were randomly primed and labeled with ³²P-dCTP Klenow incorporation

and used as probes. The northern blot hybridization and wash conditions were as follows:

hybridization in 5x SSPE, 5X Denhart's solution, 50% formamide, at 42°C followed by washing in 0.2x SSPE, 0.1% SDS, at 65°C.

5

Results of these studies revealed the transfectants expressed detectable levels of NMDAR1 and NMDAR2C mRNA of the appropriate size (based on the size of the cDNAs).

10

2. Fluorescent indicator-based assays

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Activation of ligand-gated NMDA receptors by agonists leads to an influx of cations (both monovalent and divalent), including Ca²⁺, through the receptor channel. Calcium entry into the cell through the channel can in turn induce release of calcium contained in intracellular stores. Monovalent cation entry into the cell through the channel can also result in an increase in cytoplasmic calcium levels through depolarization of the membrane and subsequent activation of voltage-dependent calcium channels. Therefore, methods of detecting transient increases in intracellular calcium concentration can be applied to the analysis of functional

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NMDA receptor expression. One method for measuring intracellular calcium levels relies on calcium-sensitive fluorescent indicators.

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Calcium-sensitive indicators, such as fluo-3 (Catalog No. F-1241, Molecular Probes, Inc., Eugene, OR) are available as acetoxymethyl esters which are membrane permeable. When the acetoxymethyl ester form of the indicator enters a cell, the ester group is removed by cytosolic esterases, thereby trapping the free indicator in the cytosol. Interaction of the free indicator with calcium results in increased fluorescence of the indicator; therefore, an increase in the intracellular Ca²⁺ concentration of cells containing the indicator can be expressed directly as an increase in fluorescence. An automated fluorescence detection system for assaying NMDA receptors has been described in commonly assigned pending US Patent Application No. 07/812,254 and corresponding PCT Patent Application No. US92/11090, incorporated by reference herein in their entirety.

Mammalian cells that have been transfected with DNA encoding NMDAR1 or NMDAR1 and NMDAR2 subunits can be analyzed for expression of

functional recombinant NMDA receptors using the automated fluorescent indicator-based assay. The assay procedure is as follows.

- Untransfected mammalian host cells (or host cells transiently transfected with pCMV-T7-2) and mammalian cells that have been transfected with NMDAR1 ± NMDAR2 subunit DNA are plated in the wells of a 96-well microtiter dish (Nunc Catalog No. 1-6708, available through Alameda Industries, Escondido, CA) that has been precoated with poly-L-lysine at a density of 2.5×10^5 cells/well and loaded with fluo-3 by incubation for 2 hours at 20°C in a medium containing 20 µM fluo-3, 0.2% Pluronic F-127 in HBS (125 mM NaCl, 5 mM KCl, 1.8 mM CaCl₂, 0.62 mM MgCl₂, 20 mM glucose, 20 mM HEPES, pH 7.4). The cells are then washed with assay buffer (i.e. HBS). The microtiter dish is then placed into a fluorescence plate reader (e.g., Fluoroskan II, Lab Products International, Ltd., Raleigh, NC) and the basal fluorescence of each well is measured and recorded before addition of 10 µM glycine and 10 µM glutamate to the wells. The fluorescence of the wells is monitored repeatedly (75 readings at 0.63-sec intervals) following addition of agonist.

The fluorescence of the untransfected host cells preferably will not change after addition of glycine and glutamate, i.e., the host cells should not express endogenous excitatory amino acid receptors. The fluorescence of mammalian cells transfected with NMDAR1 ± NMDAR2 subunit DNA will increase after addition of glycine and glutamate if a sufficient number of functional NMDA receptors are expressed at the cell surface, and fluorescence readings are taken rapidly.

The resting potential of the membrane of some mammalian host cells may be relatively positive (e.g., -35 mV). Because activation of some NMDA receptors may be significantly reduced at relatively positive potentials, it may be necessary to lower the resting potential of the membrane of cells transfected with human NMDA receptor subunit-encoding DNAs prior to assaying the cells for NMDA receptor activity using the fluorescent indicator-based assay. This may be accomplished by adding valinomycin (~10 µM) to the transfected cells prior to adding NMDA receptor agonists to initiate the assay.

3. NMDA Receptor Ligand Binding Assays

Mammalian cells transfected with NMDAR1 ± NMDAR2 subunit DNAs can be analyzed for [³H]-MK801 binding. An additional ligand-binding assay

for NMDA receptors using ^3H -CGP39653 is also described below. Rat brain membranes are included in the binding assays as a positive control.

a. Preparation of Membranes

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i. Buffy coat Homogenate from Rat Cerebral Cortex

- Buffy coat membranes are prepared from rat brain cortices as described by Jones *et al.* [(1989) *J. Pharmacol. Meth.* 21:161]. Briefly, cortices from ten freshly thawed frozen rat brains are dissected and weighed. The tissue is homogenized in 20 volumes of 0.32 M ice-cold sucrose in a glass homogenizing tube using a Teflon pestle. The suspension is centrifuged at 1,000 x g for 10 minutes at 4°C. The supernatant is decanted and centrifuged at 20,000 x g for 20 minutes at 4°C. The pellet is resuspended in 20 volumes of ice-cold distilled water with a Polytron for 30 sec at setting 6. The suspension is centrifuged at 8,000 x g for 20 minutes at 4°C. The buffy coat pellet is rinsed gently with supernatant and then recentrifuged at 48,000 x g for 20 minutes at 4°C. The pellet is resuspended in 20 volumes of ice-cold distilled water with a Polytron and centrifuged again at 48,000 x g for 20 minutes. The wash step is repeated once more. The final suspension is divided into aliquots, centrifuged. Each pellet can be stored frozen at -20°C for 12 hrs or more before use.

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ii. Membranes from Transfected and Untransfected Mammalian Cells

- In order to prepare membranes from transfected and untransfected mammalian cells, the cells are scraped from the tissue culture plates, and the plates are rinsed with 5 ml of PBS (phosphate-buffered saline: 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.7 mM KH₂PO₄). The cells are centrifuged at low speed in a table-top centrifuge, and the cell pellet is rinsed with PBS. The cell pellet is resuspended in 20 ml of 10 mM Hepes buffer, pH 7.4, using a Polytron at setting 3-6 for 30 seconds. The cell suspension is centrifuged at 48,000 x g for 20 minutes at 4°C. The supernatant is discarded, and the pellet is kept frozen for 12 hrs or more at -20°C.

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b. [³H]-MK801 Binding to NMDA Receptors

The binding of [³H]-MK801 to NMDA receptors is carried out as described by Wong *et al.* [(1986) *Proc. Natl. Acad. Sci. USA* 83:7104], with a few minor changes. Thus, on the day of the assay, the rat brain and mammalian cell (transfected and untransfected) membrane pellets are resuspended in 50 volumes of 10 mM Hepes buffer, pH 7.4, using a 10-ml syringe and a 21-gauge needle, and incubated for 20 minutes at 37°C. The supernatant is centrifuged at 48,000 x g for 20 minutes at 4°C. The pellet is resuspended in 2 ml of 10 mM Hepes, pH 7.4 and 10 centrifuged as described above. The wash step is repeated once more, and the pellet is resuspended in 10 ml of 10 mM Hepes, pH 7.4. The protein concentration is determined using the Biorad Bradford reagent. The pellet is finally resuspended in the assay buffer (10 mM Hepes, pH 7.4) at 1 mg/ml.

For binding studies, the membrane suspension is incubated in duplicate with 2.5 nM [³H]-MK801 (New England Nuclear, Boston, MA) in a total volume of 0.5 ml assay buffer (10 mM Hepes, pH 7.4) in the presence and absence of 10 µM glutamate and 10 µM glycine for 60 or 120 min at 23°C. Bound radioactivity is separated from free radioactivity by rapid filtration through Whatman GF/C filters which are presoaked for 2-3 hrs in 0.05% polyethylenimine. The filters are washed twice with 3 ml ice-cold assay buffer. The filters are dried and transferred to scintillation vials, each containing 10 ml of scintillation fluid. The vials are vortexed, and the radioactivity is measured in a Beckman scintillation counter. The nonspecific binding observed in the presence of 10 µM MK801 is subtracted from the total binding in order to determine the specific binding.

Rat brain cortical buffy coat membranes displayed specific saturable binding of [³H]-MK801. In the presence of glycine and glutamate, the ratio of total-to-nonspecific binding (S:N ratio) was 28:1, whereas in the absence of glutamate and glycine the S:N ratio was 5:1. Thus, the binding of MK801 to rat NMDA receptors is potentiated by glutamatergic agonists. Scatchard analysis of [³H]-MK801 binding to rat brain membranes indicated that the sensitivity of the assay was 90 fmoles of receptor.

c. [³H]-CGP39653 Binding to NMDA Receptors

The binding of [³H]-CGP39653 to rat brain membranes is carried out as described by Sills *et al.* [(1991) *Eur. J. Pharmacol.* 192:19]. The buffy coat membrane pellet is resuspended in 50 volumes of 5 mM Tris-HCl containing 10 mM EDTA, pH 7.7, and incubated for 10 min. at 37°C. The supernatant is centrifuged at 5 48,000 x g for 10 min. at 4°C. The wash step is repeated once and the pellet is resuspended in 10 ml of 5 mM Tris-HCl containing 10 mM EDTA, pH 7.7. This rat brain membrane suspension is incubated in duplicate or triplicate with 2.0 nM [³H]-CGP39653 (New England Nuclear) in a total volume of 0.5 ml assay buffer (5 mM Tris-HCl, pH 7.7) for 60 min at 0°C. Nonspecific binding is determined in the presence of 100 µM glutamate. Bound radioactivity is separated from the free by vacuum filtration through GF/C filters which are presoaked for 2-3 hrs in 0.05% polyethylenimine, using the filtration manifold. Unbound radioactivity is removed with two washes of 3 ml each of ice-cold buffer. The filters are dried and transferred to scintillation vials, each containing 10 ml of scintillation fluid. The vials are 10 vortexed, and the radioactivity is measured in a Beckman scintillation counter. The nonspecific binding observed in the presence of 100 µM glutamate is subtracted from the total binding to determine the specific binding.
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[³H]-CGP39653 binding was first measured as a function of membrane concentration. Specific binding increased linearly with increasing 20 membrane concentration up to 200 µg of protein in the presence of 2 nM [³H]-CGP39653.

Saturation analysis of [³H]-CGP39653 binding was carried out by incubating 150 µg of rat buffy coat homogenate with increasing concentrations of [³H]-CGP39653 for 60 min at 4°C. Scatchard analysis indicated a single class of 25 binding sites with a *B*_{max} value of 0.69 ± 0.09 pmoles/mg and a *K*_d value of 12.3 ± 0.12 nM.

While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed.
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Summary of Sequences

Sequence ID No. 1 is a nucleotide sequence encoding a human N-methyl-D-aspartate (NMDA) receptor subunit, NMDAR1A, and the deduced 35 amino acid sequence thereof.

Sequence ID No. 1A is a 3083 nucleotide sequence encoded by clone NMDA10, comprising nucleotides 320 - 3402 of Sequence ID No. 1. Thus, Sequence ID No. 1A differs from Sequence ID No. 1 in that it does not contain the 319 5' nucleotides, nor the 896 3' nucleotides thereof.

5 Sequence ID No. 1B is a 3155 nucleotide sequence encoded by clone NMDA11, comprising nucleotides 1 - 2961, plus nucleotides 3325 - 3518 of Sequence ID No. 1. Thus, Sequence ID No. 1B differs from Sequence ID No. 1 by the deletion of 363 nucleotides from the 3' portion thereof (i.e., by the deletion of nucleotides 2962 - 3324 of Sequence ID No. 1), and further by the lack of the 781 10 terminal 3' nucleotides of Sequence ID No. 1.

Sequence ID No. 1C is a 2542 nucleotide sequence encoded by clone NMDA7, comprising nucleotides 556 - 831 of Sequence ID No. 1, plus an additional 63 nucleotides (set forth in Sequence ID No. 3) and nucleotides 832 - 984, 1189 - 2961 and 3325 - 3599 of Sequence ID No. 1. Thus, Sequence ID No. 1C differs from 15 Sequence ID No. 1 in that it does not contain the 555 5'-most nucleotides thereof, it does not contain the 204 nucleotides set forth as nucleotides 985 - 1188 of Sequence ID No. 1, it does not contain the 363 3' nucleotides set forth as nucleotides 2962 - 3324 of Sequence ID No. 1, and it does not contain the 700 3'-most nucleotides of Sequence ID No. 1, while it does contain an additional 63 nucleotides (Sequence ID 20 No. 3) inserted between nucleotides 831 and 832 of Sequence ID No. 1.

Sequence ID No. 1D is a 593 nucleotide sequence encoded by clone NMDA3, comprising nucleotides 2617 - 2961, plus nucleotides 4049 - 4298 of Sequence ID No. 1. Thus, Sequence ID No. 1D differs from Sequence ID No. 1 in that it does not contain the 2616 5' nucleotides thereof, and by the deletion of 1087 25 nucleotides from the 3' portion thereof (i.e., by the deletion of nucleotides 2962 - 4048 of Sequence ID No. 1).

Sequence ID No. 1E is a nucleotide sequence encoding human NMDA receptor subunit NMDAR1- Δ 363, comprising nucleotides 1 - 2961, plus nucleotides 3325 - 4298 of Sequence ID No. 1. Thus, Sequence ID No. 1E differs from Sequence 30 ID No. 1 in that it does not contain the 363 nucleotides set forth as nucleotides 2962 - 3324 of Sequence ID No. 1.

Sequence ID No. 1F is a nucleotide sequence encoding human NMDA receptor subunit NMDAR1- Δ 1087, comprising nucleotides 1 - 2961, plus nucleotides 4049 - 4298 of Sequence ID No. 1. Thus, Sequence ID No. 1F differs from Sequence

ID No. 1 in that it does not contain the 1087 nucleotides set forth as nucleotides 2962 - 4048 of Sequence ID No. 1.

- Sequence ID No. 1G is a nucleotide sequence encoding human NMDA receptor subunit NMDAR1-I63. Sequence ID No. 1G is the same as Sequence ID No. 5 1, further comprising an additional 63 nucleotides (set forth in Sequence ID No. 3) inserted between nucleotides 831 and 832 of Sequence ID No. 1.

- Sequence ID No. 1H is a nucleotide sequence encoding human NMDA receptor subunit NMDAR1-I63-Δ204. Sequence ID No. 1H is the same as Sequence ID No. 1G, except Sequence ID No. 1H does not contain the 204 nucleotides set forth 10 as nucleotides 985 - 1188 of Sequence ID No. 1.

- Sequence ID No. 1I is a nucleotide sequence encoding human NMDA receptor subunit NMDAR1-I63-Δ204-Δ363. Sequence ID No. 1I is the same as Sequence ID No. 1H, except Sequence ID No. 1I does not contain the 363 nucleotides set forth as nucleotides 2962 - 3324 of Sequence ID No. 1.

- Sequence ID No. 1J is a nucleotide sequence encoding human NMDA receptor subunit NMDAR1-Δ204. Sequence ID No. 1J is the same as Sequence ID No. 1, except Sequence ID No. 1J does not contain the 204 nucleotides set forth as nucleotides 985 - 1188 of Sequence ID No. 1.

- Sequence ID No. 1K is a nucleotide sequence encoding human NMDA 20 receptor subunit NMDAR1-Δ204-Δ363. Sequence ID No. 1K differs from Sequence ID No. 1 in that Sequence ID No. 1K does not contain the 204 nucleotides set forth as nucleotides 985 - 1188 of Sequence ID No. 1, nor the 363 nucleotides set forth as nucleotides 2962 - 3324 of Sequence ID No. 1.

- Sequence ID No. 1L is a nucleotide sequence encoding human NMDA 25 receptor subunit NMDAR1-Δ204-Δ1087. Sequence ID No. 1L differs from Sequence ID No. 1 in that Sequence ID No. 1L does not contain the 204 nucleotides set forth as nucleotides 985 - 1188 of Sequence ID No. 1, nor the 1087 nucleotides set forth as nucleotides 2962 - 4048 of Sequence ID No. 1.

- Sequence ID No. 1M is a nucleotide sequence encoding human 30 NMDA receptor subunit NMDAR1-I63-Δ363. Sequence ID No. 1M is the same as Sequence ID No. 1G except Sequence ID No. 1M does not contain the 363 nucleotides set forth as nucleotides 2962 - 3324 of Sequence ID No. 1.

Sequence ID No. 1N is a nucleotide sequence encoding human NMDA receptor subunit NMDAR1-I63-Δ1087. Sequence No. 1N is the same as Sequence ID

No. 1G except Sequence ID No. 1N does not contain the 1087 nucleotides set forth as nucleotides 2962 - 4048 of Sequence ID No. 1.

- Sequence ID No. 1P is a nucleotide sequence encoding human NMDA receptor subunit NMDAR1-I63-Δ204-Δ1087. Sequence ID No. 1P is the same as
5 Sequence ID No. 1H, except Sequence ID No. 1P does not contain the 1087 nucleotides set forth as nucleotides 2962 - 4048 of Sequence ID No. 1.

Sequence ID No. 2 is the amino acid sequence of the NMDA receptor subunit set forth in Sequence ID No. 1.

- Sequence ID No. 2A is the amino acid sequence of a portion of an
10 NMDA receptor subunit as encoded by the nucleotide sequence of Sequence ID No. 1A.

Sequence ID No. 2B is the amino acid sequence of an NMDA receptor subunit encoded by the nucleotide sequence of Sequence ID No. 1B.

- Sequence ID No. 2C is the amino acid sequence of a portion of an
15 NMDA receptor subunit as encoded by the nucleotide sequence of Sequence ID No. 1C.

Sequence ID No. 2D is the amino acid sequence of a portion of an NMDA receptor subunit as encoded by the nucleotide sequence of Sequence ID No. 1D.

- 20 Sequence ID No. 2E is the amino acid sequence of an NMDA receptor subunit encoded by the nucleotide sequence of Sequence ID No. 1E.

Sequence ID No. 2F is the amino acid sequence of an NMDA receptor subunit encoded by the nucleotide sequence of Sequence ID No. 1F.

- Sequence ID No. 2G is the amino acid sequence of an NMDA receptor
25 subunit encoded by the nucleotide sequence of Sequence ID No. 1G.

Sequence ID No. 2H is the amino acid sequence of an NMDA receptor subunit encoded by the nucleotide sequence of Sequence ID No. 1H.

Sequence ID No. 2I is the amino acid sequence of an NMDA receptor subunit encoded by the nucleotide sequence of Sequence ID No. 1I.

- 30 Sequence ID No. 2J is the amino acid sequence of an NMDA receptor subunit encoded by the nucleotide sequence of Sequence ID No. 1J.

Sequence ID No. 2K is the amino acid sequence of an NMDA receptor subunit encoded by the nucleotide sequence of Sequence ID No. 1K.

- 35 Sequence ID No. 2L is the amino acid sequence of an NMDA receptor subunit encoded by the nucleotide sequence of Sequence ID No. 1L.

Sequence ID No. 2M is the amino acid sequence of an NMDA receptor subunit encoded by the nucleotide sequence of Sequence ID No. 1M.

Sequence ID No. 2N is the amino acid sequence of an NMDA receptor subunit encoded by the nucleotide sequence of Sequence ID No. 1N.

5 Sequence ID No. 2P is the amino acid sequence of an NMDA receptor subunit encoded by the nucleotide sequence of Sequence ID No. 1P.

Sequence ID No. 3 is a nucleotide sequence encoding the 63 nucleotide insert present in Sequence ID Nos. 1C, 1G, 1H, 1I, 1M, 1N and 1P.

10 Sequence ID No. 4 is the 21 amino acid sequence encoded by the insert set forth in Sequence ID No. 3.

Sequence ID No. 5 is a nucleotide sequence of a clone (pCMV-26-*NotI*-24) encoding a human N-methyl-D-aspartate (NMDA) receptor subunit, NMDAR2C, and the deduced amino acid sequence thereof.

15 Sequence ID No. 5A is a 2026 nucleotide sequence encoded by clone NMDA21, comprising nucleotides 931 - 2350, and 2402 - 3307 of Sequence ID No. 5. Thus, Sequence ID No. 5A differs from Sequence ID No. 5 in that it does not contain the 930 5' nucleotides thereof, nor the 51 nucleotides located at position 2351 - 2401 of Sequence ID No. 5, nor the 1061 3' nucleotides of Sequence ID No. 5.

20 Sequence ID No. 5B is a 3698 nucleotide sequence encoded by clone NMDA22, comprising nucleotides 367 - 1300 of Sequence ID No. 5, plus an additional 11 nucleotides (set forth as Sequence ID No. 9), and nucleotides 1301 - 1959 and 1975 - 4068 of Sequence ID No. 5. Thus, Sequence ID No. 5B differs from Sequence ID No. 5 by the lack of the 366 5'-most nucleotides, by the insertion of 11 nucleotides between nucleotides 1300 and 1301 of Sequence ID No. 5, and further by 25 the lack of the 15 nucleotides of Sequence ID No. 5 from residue 1960 to residue 1974.

Sequence ID No. 5C is a 3243 nucleotide sequence encoded by clone NMDA24, comprising nucleotides 861 - 1300 of Sequence ID No. 5, plus an additional 11 nucleotides (Sequence ID No. 9), nucleotides 1301 - 2350 of Sequence 30 ID No. 5, an additional 24 nucleotides (set forth as Sequence ID No. 7) and nucleotides 2351 - 4068 of Sequence ID No. 5. Thus, Sequence ID No. 5C differs from Sequence ID No. 5 in that it does not contain the 860 5'-most nucleotides thereof, while it does contain an additional 11 nucleotides (Sequence ID No. 9) inserted between nucleotides 1300 and 1301, plus an additional 24 nucleotides

(Sequence ID No. 7) inserted between nucleotides 2350 and 2351 of Sequence ID No. 5.

Sequence ID No. 5D is a 3025 nucleotide sequence encoded by clone NMDA26, comprising nucleotides 1 - 3025 of Sequence ID No. 5. Thus, Sequence 5 ID No. 5D differs from Sequence ID No. 5 in that it does not contain the 1043 3'-terminal nucleotides thereof.

Sequence ID No. 5E is a nucleotide sequence encoding human NMDA receptor subunit pCMV-26-*Scal*-24, which differs from Sequence ID No. 5 only in the insertion of 24 nucleotides (Sequence ID No. 7) between nucleotides 2350 and 10 2351 of Sequence ID No. 5.

Sequence ID No. 5F is a nucleotide sequence encoding human NMDA receptor subunit pCMV-26-*Scal*-22, which differs from Sequence ID No. 5 only in the deletion of nucleotides 1960 - 1974 of Sequence ID No. 5.

Sequence ID No. 5G is a nucleotide sequence encoding human NMDA 15 receptor subunit pCMV-26-*Scal*-21-*NotI*-24, which differs from Sequence ID No. 5 only in the deletion of nucleotides 2351 - 2401 of Sequence ID No. 5.

Sequence ID No. 5H is a nucleotide sequence encoding human NMDA receptor subunit NMDAR2C-Δ15-I24. Sequence ID No. 5H is the same as Sequence 20 ID No. 5F, except Sequence ID No. 5H further contains the 24 nucleotide insert set forth in Sequence ID No. 7, positioned between nucleotides 2350 and 2351 of Sequence ID No. 5.

Sequence ID No. 5I is a nucleotide sequence encoding human NMDA receptor subunit NMDAR2C-Δ15-Δ51. Sequence ID No. 5I is the same as Sequence 25 ID No. 5G, except Sequence ID No. 5I does not contain the 15 nucleotides set forth as nucleotides 1960 - 1974 of Sequence ID No. 5.

Sequence ID No. 6 is the amino acid sequence of the NMDA receptor subunit set forth in Sequence ID No. 5.

Sequence ID No. 6A is the amino acid sequence of a portion of an 30 NMDA receptor subunit as encoded by the nucleotide sequence of Sequence ID No. 5A.

Sequence ID No. 6B is the amino acid sequence of a portion of an NMDA receptor subunit as encoded by the nucleotide sequence of Sequence ID No. 5B.

Sequence ID No. 6C is the amino acid sequence of a portion of an NMDA receptor subunit as encoded by the nucleotide sequence of Sequence ID No. 5C.

Sequence ID No. 6D is the amino acid sequence of a portion of an
5 NMDA receptor subunit as encoded by the nucleotide sequence of Sequence ID No. 5D.

Sequence ID No. 6E is the amino acid sequence of an NMDA receptor subunit encoded by the nucleotide sequence of Sequence ID No. 5E.

Sequence ID No. 6F is the amino acid sequence of an NMDA receptor
10 subunit encoded by the nucleotide sequence of Sequence ID No. 5F.

Sequence ID No. 6G is the amino acid sequence of an NMDA receptor subunit encoded by the nucleotide sequence of Sequence ID No. 5G.

Sequence ID No. 6H is the amino acid sequence of an NMDA receptor subunit encoded by the nucleotide sequence of Sequence ID No. 5H.

15 Sequence ID No. 6I is the amino acid sequence of an NMDA receptor subunit encoded by the nucleotide sequence of Sequence ID No. 5I.

Sequence ID No. 7 is a nucleotide sequence encoding the 24 nucleotide insert present in Sequence ID Nos. 5C, 5E and 5H.

Sequence ID No. 8 is the 7 amino acid sequence encoded by
20 nucleotides 2-22 of the insert set forth in Sequence ID No. 7. Because the insert is introduced within a codon, the insert itself only encodes 7 amino acids. The terminal residues of the nucleotide insert participate in forming codons with adjacent sequence at the site of insertion.

Sequence ID No. 9 is a nucleotide sequence encoding the 11 nucleotide insert present in Sequence ID Nos. 5B and 5C.

Sequence ID No. 10 is a nucleotide sequence encoding a human N-methyl-D-aspartate (NMDA) receptor subunit, NMDAR2A.

Sequence ID No. 11 is the amino acid sequence of an NMDA receptor subunit as encoded by the nucleotide sequence set forth in Sequence ID No. 10.

30 Sequence ID No. 12 is the nucleotide sequence of 71 nucleotides of 5' untranslated sequence of clone NMDA27, plus the initiation codon (nucleotides 72 - 74) of said clone.

Sequence ID No. 13 is a nucleotide sequence of a clone encoding a human N-methyl-D-aspartate (NMDA) receptor subunit, NMDAR2B.

Sequence ID No. 14 is the amino acid sequence of the NMDA receptor subunit set forth in Sequence ID No. 13.

Sequence ID No. 15 is a nucleotide sequence of a clone encoding a human N-methyl-D-aspartate (NMDA) receptor subunit, NMDAR2D.

5 Sequence ID No. 16 is the amino acid sequence of the NMDA receptor subunit set forth in Sequence ID No. 15.

Sequence ID Nos. 17-20 are four synthetic oligonucleotides used in the preparation of an NMDAR2C clone (pCMV-26-NotI-24-GCMOD) having reduced GC nucleotide content between nucleotides 2957 and 3166.

10 Sequence ID No. 21 is the nucleotide sequence of the 195 basepair insert of NMDAR2C clone pCMV-26-NotI-24-GCMOD (replacing nucleotides 2966-3160 of Sequence ID No. 5).

SEQUENCE LISTING

(I) GENERAL INFORMATION:

5

(i) APPLICANT: Daggett, Lorrie P.

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Liaw, Chen W.

Lu, Chin-Chun

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(ii) TITLE OF INVENTION: HUMAN N-METHYL-D-ASPARTATE RECEPTOR
SUBUNITS, DNA ENCODING SAME AND USES THEREFOR

(iii) NUMBER OF SEQUENCES: 21

15

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(v) COMPUTER READABLE FORM:

25

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

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(C) CLASSIFICATION:

35

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(A) APPLICATION NUMBER: US 18 062,449

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5

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10

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(2) INFORMATION FOR SEQ ID NO:1:

15

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4298 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: both

20

(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

25

(A) NAME/KEY: CDS

(B) LOCATION: 262..3078

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

30

CAAGCCGGGC GTTCGGAGCT GTCGCCGGCC CCGCTTCAGC ACCGCGGACA GGCCCGGGCG 60

CGTGGGGCTG AGCGCGGAGC CGCGCGCAC GCTTCAGCCC CCTTCCCTC GGCGACGTC 120

CCGGGACCGC CGCTCCGGGG GASACGTGGC GTCCGCAGCC CGCGGGGCCG GGCGAGCGCA 180

35

	GGACGGCCCCG	GAAGCCCGC	GGGGATGCG	CGGAGGCC	CGCGTTCGCG	CGCGCAGAG	240
	CCAGGGCCCGC	GCCCCGAGCC	C ATG AGC ACC ATG CGC CTG CTG ACG CTC GCC				291
		Met Ser Thr Met Arg Leu Leu Thr Leu Ala					
5			1	5		10	
	CTG CTG TTC TCC TGC TCC GTC GCC CGT GCG GCG TGC GAC CCC AAG ATC						339
	Leu Leu Phe Ser Cys Ser Val Ala Arg Ala Ala Cys Asp Pro Lys Ile						
		15		20		25	
10	GTC AAC ATT GGC GCG GTG CTG AGC ACG CGG AAG CAC GAG CAG ATG TTC						387
	Val Asn Ile Gly Ala Val Leu Ser Thr Arg Lys His Glu Gln Met Phe						
		30		35		40	
15	CGC GAG GCC GTG AAC CAG GCG AAC AAG CGG CAC GGC TCC TGG AAG ATT						435
	Arg Glu Ala Val Asn Gln Ala Asn Lys Arg His Gly Ser Trp Lys Ile						
		45		50		55	
20	CAG CTC AAT GCC ACC TCC GTC ACG CAC AAG CCG AAC GCC ATC CAG ATG						483
	Gln Leu Asn Ala Thr Ser Val Thr His Lys Pro Asn Ala Ile Gln Met						
		60		65		70	
25	GCT CTG TCG GTG TGC GAG GAC CTC ATC TCC AGC CAG GTC TAC GCC ATC						531
	Ala Leu Ser Val Cys Glu Asp Leu Ile Ser Ser Gln Val Tyr Ala Ile						
		75		80		85	
						90	
	GTA GTT AGC CAT CCA CCT ACC CCC AAC GAC CAC TTC ACT CCC ACC CCT						579
	Leu Val Ser His Pro Pro Thr Pro Asn Asp His Phe Thr Pro Thr Pro						
		95		100		105	
30	GTC TCC TAC ACA GCC GGC TTC TAC CGC ATA CCC GTG CTG GGG CTG ACC						627
	Val Ser Tyr Thr Ala Gly Phe Tyr Arg Ile Pro Val Leu Gly Leu Thr						
		110		115		120	
35	ACC CGG ATG TCC ATC TAC TCG GAC AAG AGG ATG CAC CTG AGG TTC CTG						675

Thr Arg Met Ser Ile Tyr Ser Asp Lys Ser Ile His Leu Ser Phe Leu
 125 130 135

CGG ACC GTG CCG CCC TAC TCC CAC CAG TCC AGC GTG TGG TTT GAG ATG 723
 5 Arg Thr Val Pro Pro Tyr Ser His Gln Ser Ser Val Trp Phe Glu Met
 140 145 150

ATG CGT GTC TAC AGC TGG AAC CAC ATG ATC CTG CTG GTC AGC GAC GAC 771
 Met Arg Val Tyr Ser Trp Asn His Ile Ile Leu Val Ser Asp Asp
 10 155 160 165 170

CAC GAG GGC CGG GCG GCT CAG AAA CGC CTG GAG ACG CTG CTG GAG GAG 819
 His Glu Gly Arg Ala Ala Gln Lys Arg Leu Glu Thr Leu Leu Glu Glu
 175 180 185

15 CGT GAG TCC AAG GCA GAG AAG GTG CTG CAG TTT GAC CCA GGG ACC AAG 867
 Arg Glu Ser Lys Ala Glu Lys Val Leu Gln Phe Asp Pro Gly Thr Lys
 190 195 200

20 AAC GTG ACG GCC CTG CTG ATG GAG GCG AAA GAG CTC GAG GCC CGG GTC 915
 Asn Val Thr Ala Leu Leu Met Glu Ala Lys Glu Leu Glu Ala Arg Val
 205 210 215

ATC ATC CTT TCT GCC AGC GAG GAC GAT GCT GCC ACT GTA TAC CGC GCA 963
 25 Ile Ile Leu Ser Ala Ser Glu Asp Asp Ala Ala Thr Val Tyr Arg Ala
 220 225 230

GCC GCG ATG CTG AAC ATG ACG GGC TCC GGG TAC GTG TGG CTG GTC GGC 1011
 Ala Ala Met Leu Asn Met Thr Gly Ser Gly Tyr Val Trp Leu Val Gly
 30 235 240 245 250

GAG CGC GAG ATC TCG GGG AAC GCC CTG CGC TAC GCC CCA GAC GGC ATC 1059
 Glu Arg Glu Ile Ser Gly Asn Ala Leu Arg Tyr Ala Pro Asp Gly Ile
 255 260 265

	CTC GGG CTG CAG CTC ATC AAC GGC AAG AAC GAG TCG GCC CAC ATC AGC		1107	
	Leu Gly Leu Gln Leu Ile Asn Gly Lys Asn Glu Ser Ala His Ile Ser			
	271	275	280	
5	GAC GCC GTG GGC GTG GTG GCC CAG GCC GTG CAC GAG CTC CTC GAG AAG		1165	
	Asp Ala Val Gly Val Val Ala Gln Ala Val His Glu Leu Leu Glu Lys			
	285	290	295	
	SAG AAC ATC ACC GAC CCG CCG CGG GGC TGC GTG GGC AAC ACC AAC ATC		1203	
10	Glu Asn Ile Thr Asp Pro Pro Arg Gly Cys Val Gly Asn Thr Asn Ile			
	300	305	310	
	TGG AAG ACC GGG CCG CTC TTC AAG AGA GTG CTG ATG TCT TCC AAG TAT		1251	
	Trp Lys Thr Gly Pro Leu Phe Lys Arg Val Leu Met Ser Ser Lys Tyr			
15	315	320	325	330
	GCG GAT GGG GTG ACT GGT CGC GTG GAG TTC AAT GAG GAT GGG GAC CGG		1299	
	Ala Asp Gly Val Thr Gly Arg Val Glu Phe Asn Glu Asp Gly Asp Arg			
	335	340	345	
20	AAG TTC GGC AAC TAC AGC ATC ATG AAC CTG CAG AAC CGC AAG CTG GTG		1347	
	Cys Phe Ala Asn Tyr Ser Ile Met Asn Leu Gln Asn Arg Lys Leu Val			
	350	355	360	
25	CAA GTG GGC ATC TAC AAT GGC ACC CAC GTC ATC CCT AAT GAC AGG AAG		1395	
	Gln Val Gly Ile Tyr Asn Gly Thr His Val Ile Pro Asn Asp Arg Lys			
	365	370	375	
	ATC ATC TGG CCA GGC GGA GAG ACA GAG AAG CCT CGA GGG TAC CAG ATG		1443	
30	Ile Ile Trp Pro Gly Gly Glu Thr Glu Lys Pro Arg Gly Tyr Gln Met			
	380	385	390	
	TCC ACC AGA CTG AAG ATT GTG ACG ATC CAC CAG GAG CCC TTC GTG TAC		1491	
	Ser Thr Arg Leu Lys Ile Val Thr Ile His Gln Glu Pro Phe Val Tyr			
35	395	400	405	410

	GTC AAG CCC ACG CTG AGT GAT GGG ACA TGC AAG GAG GAG TTC ACA GTC	1539		
	Val Lys Pro Thr Ile Ser Asp Gly Thr Cys Lys Glu Glu Phe Thr Val			
	415	420	425	
5				
	AAC GGC GAC CCA GTC AAG AAG GTG ATC TGC ACC GGG CCC AAC GAC ACG	1567		
	Asn Gly Asp Pro Val Lys Lys Val Ile Cys Thr Gly Pro Asn Asp Thr			
	430	435	440	
10	TGC CGG GGC AGC CCC CGC CAC ACG GTG CCT CAG TGT TGC TAC GGC TTT	1635		
	Ser Pro Gly Ser Pro Arg His Thr Val Pro Gln Cys Cys Tyr Gly Phe			
	445	450	455	
	TGC ATC GAC CTG CTC ATC AAG CTG GCA CGG ACC ATG AAC TTC ACC TAC	1683		
15	Cys Ile Asp Leu Leu Ile Lys Leu Ala Arg Thr Met Asn Phe Thr Tyr			
	460	465	470	
	GAG CTG CAC CTG GTG GCA GAT GGC AAG TTC GGC ACA CAG GAG CGG GTG	1731		
	Gly Val His Leu Val Ala Asp Gly Lys Phe Gly Thr Gln Gly Arg Val			
20	475	480	485	490
	AAC AAC AGC AAC AAG AAG GAG TGG AAT GGG ATG ATG GGC GAG CTG CTC	1779		
	Asn Asn Ser Asn Lys Lys Glu Trp Asn Gly Met Met Gly Glu Leu Leu			
	495	500	505	
25				
	AGC GGG CAG GCA GAC ATG ATC GTG GCG CCG CTA ACC ATA AAC AAC GAG	1827		
	Ser Gly Gln Ala Asp Met Ile Val Ala Pro Leu Thr Ile Asn Asn Glu			
	510	515	520	
30	CGC GCG CAG TAC ATC GAG TTT TCC AAG CCC TTC AAG TAC CAG GGC CTG	1875		
	Arg Ala Gln Tyr Ile Glu Phe Ser Lys Pro Phe Lys Tyr Gln Gly Leu			
	525	530	535	
	ACT ATT CTG GTC AAG AAG GAG ATT CCC CGG AGC ACG CTG GAC TCG TTC	1923		
35	Thr Ile Leu Val Lys Lys Glu Ile Pro Arg Ser Thr Leu Asp Ser Phe			

	541	545	550	
	ATG CAG CCG TTC CAG AGC ACA CTG TGG CTG CTG GTG GGG CTG TCG GTG			1971
	Met Gln Pro Phe Gln Ser Thr Leu Trp Leu Leu Val Gly Leu Ser Val			
5	555	560	565	570
	CAC GTG GTG GCC GTG ATG CTG TAC CTG CTG GAC CGG TPC AGC CCC TPC			2019
	His Val Val Ala Val Met Leu Tyr Leu Leu Asp Arg Phe Ser Pro Phe			
	575	580	585	
10				
	GGC CGG TTC AAG GTG AAC AGC GAG GAG GAG GAG GAC GCA CTG ACC			2067
	Gly Arg Phe Lys Val Asn Ser Glu Glu Glu Glu Asp Ala Leu Thr			
	590	595	600	
15				
	CTG TCC TCG GCC ATG TGG TTC TCC TGG GGC GTC CTG CTC AAC TCC GGC			2115
	Leu Ser Ser Ala Met Trp Phe Ser Trp Gly Val Leu Leu Asn Ser Gly			
	605	610	615	
	ATC GGG GAA GGC GCC CCC AGA AGC TTC TCA GCG CGC ATC CTG GGC ATG			2163
20	Ile Gly Glu Gly Ala Pro Arg Ser Phe Ser Ala Arg Ile Leu Gly Met			
	620	625	630	
	GTG TGG GCC GGC TTT GCC ATG ATC ATC GTG GCC TCC TAC ACC GCC AAC			2211
	Val Trp Ala Gly Phe Ala Met Ile Ile Val Ala Ser Tyr Thr Ala Asn			
25	635	640	645	650
	CTG GCG GCC TTC CTG GTG CTG GAC CGG CGG GAG GAG CGC ATC ACG GGC			2259
	Leu Ala Ala Phe Leu Val Leu Asp Arg Pro Glu Glu Arg Ile Thr Gly			
	655	660	665	
30				
	ATC AAC GAC CCT CGG CTG AGG AAC CCC TCG GAC AAG TTT ATC TAC GGC			2307
	Ile Asn Asp Pro Arg Leu Arg Asn Pro Ser Asp Lys Phe Ile Tyr Ala			
	670	675	680	
35	ACG GTG AAG CAG AGC TCC GTG GAT ATC TAC TTC CGG CGC CAG GTG GAG			2355

	Thr Val Lys Gln Ser Ser Val Asp Ile Tyr Phe Arg Arg Gln Val Glu			
	685	691	695	
	CTG AGC ACC ATG TAC CGG CAT ATG GAG AAG CAT AAC TAC GAG AGT GCG			2403
5	Leu Ser Thr Met Tyr Arg His Met Glu Lys His Asn Tyr Glu Ser Ala			
	700	705	710	
	CGG GAG GCC ATC CAG GCG GTG AGA GAC AAC AAG CTG CAT GCC TTC ATC			2451
	Ala Glu Ala Ile Gln Ala Val Arg Asp Asn Lys Leu His Ala Phe Ile			
10	715	720	725	730
	TGG GAC TCG GCG GTG CTG GAG TTC GAG GCC TCG CAG AAG TGC GAC CTG			2499
	Trp Asp Ser Ala Val Leu Glu Phe Glu Ala Ser Gln Lys Cys Asp Leu			
	735	740	745	
15				
	GTG ACG ACT GGA GAG CTG TTT TTC CGC TCG GGC TTC GGC ATA GGC ATG			2547
	Val Thr Thr Gly Glu Leu Phe Phe Arg Ser Gly Phe Gly Ile Gly Met			
	750	755	760	
20	CGC AAA GAC AGC CCC TGG AAG CAG AAC GTC TCC CTG TCC ATC CTC AAG			2595
	Arg Lys Asp Ser Pro Trp Lys Gln Asn Val Ser Leu Ser Ile Leu Lys			
	765	770	775	
	TCC CAC GAG AAT GGC TTC ATG GAA GAC CTG GAC AAG ACG TGG GTT CGG			2643
25	Ser His Glu Asn Gly Phe Met Glu Asp Leu Asp Lys Thr Trp Val Arg			
	780	785	790	
	TAT CAG GAA TGT GAC TCG CGC AGC AAC GGC CCT GCG ACC CTT ACT TTT			2691
	Tyr Gln Glu Cys Asp Ser Arg Ser Asn Ala Pro Ala Thr Leu Thr Phe			
30	795	800	805	810
	GAG AAC ATG GCC GGG GTC TTC ATG CTG GTA GCT GGG GGC ATC GTG GCC			2732
	Glu Asn Met Ala Gly Val Phe Met Leu Val Ala Gly Gly Ile Val Ala			
	815	820	825	

	GGG ATC TTC CTG ATT TTC ATC GAG ATT GCC TAC AAG CGG CAC AAG GAT	830	835	840	2787
	Gly Ile Phe Leu Ile Phe Ile Glu Ile Ala Tyr Lys Arg His Lys Asp				
5	GCT CGC CGG AAG CAG ATG CAG CTG GGC TTT GGC GGC GTT AAC GTG TGG	845	850	855	2835
	Ala Arg Arg Lys Gln Met Gln Leu Ala Phe Ala Ala Val Asn Val Trp				
10	CGG AAG AAC CTG CAG GAT AGA AAG AGT GGT AGA GCA GAG CCT SAC CCT	860	865	870	2883
	Arg Lys Asn Leu Gln Asp Arg Lys Ser Gly Arg Ala Glu Pro Asp Pro				
15	AAA AAG AAA GCC ACA TTT AGG GCT ATC ACC TCC ACC CTG GCT TCC AGC	875	880	885	2931
	Lys Lys Lys Ala Thr Phe Arg Ala Ile Thr Ser Thr Leu Ala Ser Ser				
20	TTC AAG AGG CGT AGG TCC TCC AAA GAC ACG AGC ACC GGG GGT GGA CGC	895	900	905	2979
	Phe Lys Arg Arg Ser Ser Lys Asp Thr Ser Thr Gly Gly Arg				
25	GGT GCT TTG CAA AAC CAA AAA GAC ACA GTG CTG CGG CGA CGC GCT ATT	910	915	920	3027
	Gly Ala Leu Gln Asn Gln Lys Asp Thr Val Leu Pro Arg Arg Ala Ile				
30	GAG AGG GAG GAG GGC CAG CTG CAG CTG TGT TCC CGT CAT AGG GAG AGC	925	930	935	3075
	Glu Arg Glu Glu Gly Gln Leu Gln Leu Cys Ser Arg His Arg Glu Ser				
35	TGAGACTCCC CGCGCGCGCT CCTCTGCGCC CGCCCCCGCA GACAGACAGA CAGACGGACG	940	945	950	3135
	GGACAGCGGGC CGGGCGCACG CAGAGCGCG GAGCACCAAG GGGTGGGGG AGGAGCACCC				
	CGAGCGCTCC CGAGGCGTCCG CCTGCCGCC CGCGCGTTGG CGGGCTGGCC GGTCCACCCC				
40	GTCCCCGGCC CGCGCGCTCC CGAGCGCTCC CGCTAACGGG CGCCTTGCT GTGTATTCT	955	960	965	3195
45					

	ATTTTGAGC AGTACCATCC CACTGATATC ACGGGCCCCC TCAACCTCTC AGATCCTCG	3375
	GTCAGAACCG TGCTGTGAGG CCCCGGGAGG CGCCCACTG CCCAGTTAGC CGGGCCAAGG	3435
5	ACACTGATGG GTGCTGCTGC TCGGGAAAGG CTGAGGGAAAG CCCACCGGCC CCAGAGACTG	3495
	CCCACCCGGG GCGTCCCGTC CGTCCCGGGG CCCACCCGGG TGCTGGGGG GCAGCCCCTG	3555
10	CTGGACCAAAG GPGGGGACCG GAGGGCTGA GGACGGGGCA GAGCTGAATC GGCTGGGAG	3615
	GGCCCGAGGG CGCTCGGGCA GAGGCAGGGC CCTGGGGCT CTGAGGAGTG GGGAGGGGG	3675
	GCTAACTGCC CCCAGGGGAA GGGGCTTGGA GCAGAGACGG CAGCCOCATC CTCCCGCAG	3735
15	CAACAGCTG ACCCACAGTG GGGCCATGG CCCAGCTGG CTGGGTGGC CCTCTCGGG	3795
	CGCTGCGCT CCTCTGAGC CPGAGCTCCA CCTCCCGCTC TTCTTGCGC ACGGCCACC	3855
20	AAACACCCCG TCTGGCCCTT GACGCCACAC GCGGGGGCTG GCCTGCCTT CCCAACGGC	3915
	CGTCCCTGAC TTCCGAGCTG CGAGGGCTC CGCGCGCTC GGCGCGCTC CTCCAGAATC	3975
	GAGAGGGCTG ACGCCCTCCT CGCTCTGCG ACAGAAAGGG GCGTCCCGGG	4035
25	GGGTCCCCGG AGGCTGGCTC GGGACTGTCT TCAACCTGC CCTGCACCTT GGGCACGGGA	4095
	GAGCGCCACC CGGGGGGGCC CGCGCTCGCT CGGGGTGGT GACGGGGCGG CGACCTTGTA	4155
30	CAGAACCCAGC ACTCCGAGGG CGCGAGGGG TGCTTGGCC GTGGCGAGCT GCGCTCTGCC	4215
	CCTCGCTCCC CAGGGTGCAG GCGCGCACCG CCCAACGGG ACCTCCGGT GATGCAGTG	4275
	GTGATGCATA AAGGAATGTC ACG	4298
35		

(2) INFORMATION FOR SEQ ID NO:2:

(1) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 936 amino acids
 (B) TYPE: amino acid
 (C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ser Thr Met Arg Leu Leu Thr Leu Ala Leu Leu Phe Ser Cys Ser

1 5 10 15

15 Val Ala Arg Ala Ala Cys Asp Pro Lys Ile Val Asn Ile Gly Ala Val

20 25 30 35 40 45

No. 102-1142 Arg. Mar. 18, Sess. Term, 1992, Ill. Gen. Law. Ass., 102d Gen. Assem., 1st Reg. Sess.

55 60

25 Val Thr His Lys Pro Asn Ala Ile Gln Met Ala Leu Ser Val Cys Glu
65 70 75 80

Asp Leu Ile Ser Ser Gln Val Tyr Ala Ile Leu Val Ser His Pro Pro
85 90 95

30 Thr Pro Asn Asp His Phe Thr Pro Thr Pro Val Ser Tyr Thr Ala Gly
 100 105 110

Phe Tyr Arg Ile Pro Val Leu Gly Leu Thr Thr Arg Met Ser Ile Tyr

35 115 120 125

Ser Asp Lys Ser Ile His Leu Ser Phe Leu Arg Thr Val Pro Pro Tyr
130 135 140

5 Ser His Gln Ser Ser Val Trp Phe Glu Met Met Arg Val Tyr Ser Trp
145 150 155 160

Asn His Ile Ile Leu Leu Val Ser Asp Asp His Glu Gly Arg Ala Ala
165 170 175

10 Gin Lys Arg Leu Glu Thr Leu Leu Glu Glu Arg Glu Ser Lys Ala Glu
180 185 190

Lys Val Leu Gln Phe Asp Pro Gly Thr Lys Asn Val Thr Ala Leu Leu
195 200 205

Met Glu Ala Lys Glu Leu Glu Ala Arg Val Ile Ile Leu Ser Ala Ser
210 215 220

20 Glu Asp Asp Ala Ala Thr Val Tyr Arg Ala Ala Ala Met Leu Asn Met
225 230 235 240

Thr Gly Ser Gly Tyr Val Trp Leu Val Gly Glu Arg Glu Ile Ser Gly
245 250 255

25 Asn Ala Leu Arg Tyr Ala Pro Asp Gly Ile Leu Gly Leu Gln Leu Ile
260 265 270

Asn Gly Lys Asn Glu Ser Ala His Ile Ser Asp Ala Val Gly Val Val
275 280 285

Ala Gln Ala Val His Glu Leu Leu Glu Lys Glu Asn Ile Thr Asp Pro
290 295 300

35 Pro Arg Gly Cys Val Gly Asn Thr Asn Ile Trp Lys Thr Gly Pro Leu

	305	310	315	320
	Phe Lys Arg Val Leu Met Ser Ser Lys Tyr Ala Asp Gly Val Thr Gly			
	325	330	335	
5	Arg Val Glu Phe Asn Glu Asp Gly Asp Arg Lys Phe Ala Asn Tyr Ser			
	340	345	350	
	Ile Met Asn Leu Gln Asn Arg Lys Leu Val Gln Val Gly Ile Tyr Asn			
10	355	360	365	
	Gly Thr His Val Ile Pro Asn Asp Arg Lys Ile Ile Trp Pro Gly Gly			
	370	375	380	
15	Glu Thr Glu Lys Pro Arg Gly Tyr Gln Met Ser Thr Arg Leu Lys Ile			
	385	390	395	400
	Val Thr Ile His Gln Glu Pro Phe Val Tyr Val Lys Pro Thr Leu Ser			
	405	410	415	
20	Asp Gly Thr Cys Lys Glu Glu Phe Thr Val Asn Gly Asp Pro Val Lys			
	420	425	430	
	Lys Val Ile Cys Thr Gly Pro Asn Asp Thr Ser Pro Gly Ser Pro Arg			
25	435	440	445	
	His Thr Val Pro Gln Cys Cys Tyr Gly Phe Cys Ile Asp Leu Leu Ile			
	450	455	460	
30	Lys Leu Ala Arg Thr Met Asn Phe Thr Tyr Glu Val His Leu Val Ala			
	465	470	475	480
	Asp Gly Lys Phe Gly Thr Gln Glu Arg Val Asn Asn Ser Asn Lys Lys			
	485	490	495	
35				

Glu Trp Asn Gly Met Met Gly Glu Leu Leu Ser Gly Gln Ala Asp Met
 500 515 515
 Ile Val Ala Pro Leu Thr Ile Asn Asn Glu Arg Ala Gln Tyr Ile Glu
5 515 520 525
 Phe Ser Lys Pro Phe Lys Tyr Gln Gly Leu Thr Ile Leu Val Lys Lys
 530 535 540
10 Glu Ile Pro Arg Ser Thr Leu Asp Ser Phe Met Gln Pro Phe Gln Ser
 545 550 555 560
 Thr Leu Trp Leu Leu Val Gly Leu Ser Val His Val Val Ala Val Met
 565 570 575
15
 Leu Tyr Leu Leu Asp Arg Phe Ser Pro Phe Gly Arg Phe Lys Val Asn
 580 585 590
 Ser Glu Glu Glu Glu Asp Ala Leu Thr Leu Ser Ser Ala Met Trp
20 595 600 605
 Phe Ser Trp Gly Val Leu Leu Asn Ser Gly Ile Gly Glu Gly Ala Pro
 610 615 620
25 Arg Ser Phe Ser Ala Arg Ile Leu Gly Met Val Trp Ala Gly Phe Ala
 625 630 635 640
 Met Ile Ile Val Ala Ser Tyr Thr Ala Asn Leu Ala Ala Phe Leu Val
 645 650 655
30
 Leu Asp Arg Pro Glu Glu Arg Ile Thr Gly Ile Asn Asp Pro Arg Leu
 660 665 670
 Arg Asn Pro Ser Asp Lys Phe Ile Tyr Ala Thr Val Lys Gln Ser Ser
35 675 680 685

Val Asp Ile Tyr Phe Arg Arg Gln Val Glu Leu Ser Thr Met Tyr Arg

690 695 700

5 His Met Glu Lys His Asn Tyr Glu Ser Ala Ala Glu Ala Ile Gln Ala
705 710 715 720

Val Arg Asp Asn Lys Leu His Ala Phe Ile Trp Asp Ser Ala Val Leu
725 730 735

10 Glu Phe Glu Ala Ser Gln Lys Cys Asp Leu Val Thr Thr Gly Glu Leu
740 745 750

Phe Phe Arg Ser Gly Phe Gly Ile Gly Met Arg Lys Asp Ser Pro Trp
15 755 760 765

Lys Gln Asn Val Ser Leu Ser Ile Leu Lys Ser His Glu Asn Gly Phe
770 775 780

20 Met Glu Asp Leu Asp Lys Thr Trp Val Arg Tyr Gln Glu Cys Asp Ser
785 790 795 800

Arg Ser Asn Ala Pro Ala Thr Leu Thr Phe Glu Asn Met Ala Gly Val
805 810 815

25 Phe Met Leu Val Ala Gly Gly Ile Val Ala Gly Ile Phe Leu Ile Phe
820 825 830

Ile Glu Ile Ala Tyr Lys Arg His Lys Asp Ala Arg Arg Lys Gln Met
30 835 840 845

Gln Leu Ala Phe Ala Ala Val Asn Val Trp Arg Lys Asn Leu Gln Asp
850 855 860

35 Arg Lys Ser Gly Arg Ala Glu Pro Asp Pro Lys Lys Lys Ala Thr Phe

865 870 875 880

Arg Ala Ile Thr Ser Thr Leu Ala Ser Ser Phe Lys Arg Arg Arg Ser

885 890 895

5

Ser Lys Asp Thr Ser Thr Gly Gly Arg Gly Ala Leu Gln Asn Gln

900 905 910

Lys Asp Thr Val Leu Pro Arg Arg Ala Ile Glu Arg Glu Glu Gly Gln

10 915 920 925

Leu Gln Leu Cys Ser Arg His Arg Glu Ser

930 935

15

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 63 base pairs

20 (B) TYPE: nucleic acid

(C) STRANDEDNESS: both

(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

25

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..63

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGT AAA AAA AGG AAC TAT GAA AAC CTC GAC CAA CTG TCC TAT GAC AAC 48

Ser Lys Lys Arg Asn Tyr Glu Asn Leu Asp Gln Leu Ser Tyr Asp Asn

1 5 10 15

35

AAG CGC GGA CCC AAG

63

Lys Arg Gly Pro Lys

20

5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ser Lys Lys Arg Asn Tyr Glu Asn Leu Asp Gln Leu Ser Tyr Asp Asn

1 5 10 15

20

Lys Arg Gly Pro Lys

20

(2) INFORMATION FOR SEQ ID NO:5:

25

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4340 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: both

30

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

35

- (A) NAME KEY: CDS

(B) LOCATION: 189..3899

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

5	CCTTTAATAA GATTTGCCAC GTACACTGGA GCCATCGCGA GTGTCCCTGAA GCGCGGGGTG	60
	ACGGTGGCTC TCGCTGCTCG CGCGCGCTGC TCCCGCGGGG GGAGGCTGAT GCCACGTTGC	120
	CTATGAATTAA TTATCGCG CGCTAAAAAT ACCCGAACT TCACAGCGCG AGTGAACCGC	180
10	CGGTGGAC ATG GGT GGG GCC CTG GGG CCG GCG CTG TTG CTC ACG TCG CTC	230
	Met Gly Ala Leu Gly Pro Ala Leu Leu Leu Thr Ser Leu	
	1 5 10	
15	TTC GGT GCC TGG GCA GGG CTG GGT CCG GGG CAG GGC GAG CAG GGC ATG	278
	Phe Gly Ala Trp Ala Gly Leu Gly Pro Gly Gln Gly Glu Gln Gly Met	
15 20 25 30		
	ACG GTG GCC GTG GTG TTT AGC AGC TCA GGG CCG CCC CAG GGC CAG TTC	326
20	Thr Val Ala Val Val Phe Ser Ser Ser Gly Pro Pro Gln Ala Gln Phe	
	35 40 45	
	CGT GTC CGC CTC ACG CCC CAG AGC TTC CTG GAC CTA CCC CTG GAG ATC	374
	Arg Val Arg Leu Thr Pro Gln Ser Phe Leu Asp Leu Pro Leu Glu Ile	
25 50 55 60		
	CAG CCG CTC ACA GTT GGG GTC AAC ACC ACC AAC CCC AGC AGC CTC CTC	422
	Gln Pro Leu Thr Val Gly Val Asn Thr Thr Asn Pro Ser Ser Leu Leu	
	65 70 75	
30	ACC CAG ATC TGC GGC CTC CTG GGT GCT GCC CAC GTC CAC GGC ATT GTC	470
	Thr Gln Ile Cys Gly Leu Leu Gly Ala Ala His Val His Gly Ile Val	
	80 85 90	
35	TTT GAG GAC AAC GTG GAC ACC GAG GCG GTG GCG CAG ATC CTT GAC TTC	518

Phe Glu Asp Asn Val Asp Thr Glu Ala Val Ala Gln Ile Leu Asp Phe
 95 100 105 110

ATC TCC TCC CAG ACC CAT GTG CGT ATC CTC AGC ATC AGC GGA GGC TCT 566
 5 Ile Ser Ser Gln Thr His Val Pro Ile Leu Ser Ile Ser Gly Gly Ser
 115 120 125

GCT GTG GTC CTC ACC CCC AAG GAG CGG GGC TCC GCC TTC CTG CAG CTG 614
 Ala Val Val Leu Thr Pro Lys Gln Pro Gly Ser Ala Phe Leu Gln Leu
 10 130 135 140

GCG GTG TCC CTG GAG CAG CAG CTG CAG GTG CTG TTC AAG GTG CTG GAA 662
 Gly Val Ser Leu Glu Gln Gln Leu Gln Val Leu Phe Lys Val Leu Glu
 145 150 155

15 GAG TAC GAC TGG AGC GCC TTC GCG GTC ATC ACC AGC CTG CAC CGG GGC 710
 Gln Tyr Asp Trp Ser Ala Phe Ala Val Ile Thr Ser Leu His Pro Gly
 160 165 170

20 CAC GCG CTC TTC CTG GAG GGC GTG CGC GCG GTC GCC GAC GCG AGC CAC 758
 His Ala Leu Phe Leu Glu Gly Val Arg Ala Val Ala Asp Ala Ser His
 175 180 185 190

GTG AGT TGG CGG CTG CTG GAC GTG GTC ACG CTG GAA CTG GAC CGG GGA 806
 25 Val Ser Trp Arg Leu Leu Asp Val Val Thr Leu Glu Leu Asp Pro Gly
 195 200 205

GGG CCG CGC GCG CGC ACG CAG CGC CTG CTG CGC CAG CTC GAC GCG CCC 854
 Gly Pro Arg Ala Arg Thr Gln Arg Leu Leu Arg Gln Leu Asp Ala Pro
 30 210 215 220

GTG TTT GTG GCC TAC TGC TCG CGG GAG GAG GCG GAG GTG CTC TTC GCC 902
 Val Phe Val Ala Tyr Cys Ser Arg Gln Glu Ala Glu Val Leu Phe Ala
 225 230 235

35

	GAG GCG GCG CAG GCC GGT CTG CTG GGG CCC GGC CAC GTG TGG CTG GTG	950
	Glu Ala Ala Gln Ala Gly Leu Val Gly Pro Gly His Val Trp Leu Val	
	240 245 250	
5	CCC AAC CTG GCG CTG GGC AGC ACC GAT GCG CCC CCC GGC ACC TTC CCC	998
	Pro Asn Leu Ala Leu Gly Ser Thr Asp Ala Pro Pro Ala Thr Phe Pro	
	255 260 265 270	
	GTG GGC CTC ATC AGC GTC GTC ACC GAG AGC TGG CGC CTC AGC CTG CGC	1046
10	Val Gly Leu Ile Ser Val Val Thr Glu Ser Trp Arg Leu Ser Leu Arg	
	275 280 285	
	CAG AAG GTG CGC GAC GGC GTG GCC ATT CTG GCC CTG GGC GGC CAC AGC	1094
	Gln Lys Val Arg Asp Gly Val Ala Ile Leu Ala Leu Gly Ala His Ser	
15	290 295 300	
	TAC TGG CGC CAG CAT GGA ACC CTG CCA GCC CGG GGC GAG TGC CGT	1142
	Tyr Trp Arg Gln His Gly Thr Leu Pro Ala Pro Ala Gly Asp Cys Arg	
	305 310 315	
20	GTT CAC CCT GGG CCC GTC AGC CCT GCC CGG GAG GCC TTC TAC AGG CAC	1190
	Val His Pro Gly Pro Val Ser Pro Ala Arg Glu Ala Phe Tyr Arg His	
	320 325 330	
25	CTA CTG AAT GTC ACC TGG GAG GGC CGA GAC TTC TCC AGC CCT GGT	1238
	Leu Leu Asn Val Thr Trp Glu Gly Arg Asp Phe Ser Phe Ser Pro Gly	
	335 340 345 350	
	GGG TAC CTG GTC CAG CCC ACC ATG GTG GTG ATC GCC CTC AAC CGG CAC	1286
30	Gly Tyr Leu Val Gln Pro Thr Met Val Val Ile Ala Leu Asn Arg His	
	355 360 365	
	CGC CTC TGG GAG ATG GTG GGG CGC TGG GAG CAT GGC GTC CTA TAC ATG	1334
	Arg Leu Trp Glu Met Val Gly Arg Trp Glu His Gly Val Leu Tyr Met	
35	370 375 380	

	AAG TAC CCC GTG TGG CCT CGC TAC AGT GGC TCT CTG CAG CCT GTG GTG	1382
	Lys Tyr Pro Val Trp Pro Arg Tyr Ser Ala Ser Leu Gln Pro Val Val	
	385 390 395	
5		
	GAC AGT CGG CAC CTG ACG GTG GCC ACG CTG GAA GAG CGG CCC TTT GTC	1430
	Asp Ser Arg His Leu Thr Val Ala Thr Leu Glu Glu Arg Pro Phe Val	
	400 405 410	
10	ATC GTG GAG AGC CCT GAC CCT GGC ACA GGA GGC TGT GTC CCC AAC ACC	1478
	Ile Val Glu Ser Pro Asp Pro Gly Thr Gly Gly Cys Val Pro Asn Thr	
	415 420 425 430	
	GTG CCC TGC CGC AGG CAG AGC AAC CAC ACC TTC AGC AGC GGG GAC GTG	1526
15	Val Pro Cys Arg Arg Gln Ser Asn His Thr Phe Ser Ser Gly Asp Val	
	435 440 445	
	GCC CCC TAC ACC AAG CTC TGC TGT AAG GGA TTC TGC ATC GAC ATC CTC	1574
	Ala Pro Tyr Thr Lys Leu Cys Cys Lys Gly Phe Cys Ile Asp Ile Leu	
20	450 455 460	
	AAG AAG CTG GCG AGA GTG GTC AAA TTC TCC TAC GAC CTG TAC CTG GTG	1622
	Lys Lys Leu Ala Arg Val Val Lys Phe Ser Tyr Asp Leu Tyr Leu Val	
	465 470 475	
25		
	ACC AAC GGC AAG CAT GGC AAG CGG GTG CGC GGC GTA TGG AAC GGC ATG	1670
	Thr Asn Gly Lys His Gly Lys Arg Val Arg Gly Val Trp Asn Gly Met	
	480 485 490	
30	ATT GGG GAG GTG TAC TAC AAG CGG GCA GAC ATG GGC ATC GGC TCC CTC	1718
	Ile Gly Glu Val Tyr Tyr Lys Arg Ala Asp Met Ala Ile Gly Ser Leu	
	495 500 505 510	
	ACC ATC AAT GAG GAA CGC TCC GAG ATC GTA GAT TTC TCT GTA CCC TTT	1766
35	Thr Ile Asn Glu Glu Arg Ser Glu Ile Val Asp Phe Ser Val Pro Phe	

	515	520	525	
				1814
	GTG GAG ACG GGC ATC AGT GTG ATG GTG GCT CGC AGC AAT GGC ACC GTC Val Glu Thr Gly Ile Ser Val Met Val Ala Arg Ser Asn Gly Thr Val			
5	530	535	540	
				1862
	TCC CCC TCG GGC TTC TTG GAG CCA TAT AGC CCT GCA GTG TGG GTG ATG Ser Pro Ser Ala Phe Leu Glu Pro Tyr Ser Pro Ala Val Trp Val Met			
	545	550	555	
10				
				1910
	ATG TTT GTC ATG TGC CTC ACT GTG GTG GCC ATC ACC GTC TTC ATG TTC Met Phe Val Met Cys Leu Thr Val Val Ala Ile Thr Val Phe Met Phe			
	560	565	570	
15				
				1958
	GAG TAC TTC AGC CCT GTC AGC TAC AAC CAG AAC CTC ACC AGA GGC AAG Glu Tyr Phe Ser Pro Val Ser Tyr Asn Gln Asn Leu Thr Arg Gly Lys			
	575	580	585	590
				2006
20	AAG TCC GGG GGC CCA GCT TTC ACT ATC GGC AAG TCC GTG TGG CTG CTG Lys Ser Gly Pro Ala Phe Thr Ile Gly Lys Ser Val Trp Leu Leu			
	595	600	605	
				2054
	TGG GCG CTG GTC TTC AAC AAC TCA GTG CCC ATC GAG AAC CGG CGG GGC Trp Ala Leu Val Phe Asn Asn Ser Val Pro Ile Glu Asn Pro Arg Gly			
25	610	615	620	
				2102
	ACC ACC AGC AAG ATC ATG GTT CTG GTC TGG GGC TTC TTT GCT GTC ATC Thr Thr Ser Lys Ile Met Val Leu Val Trp Ala Phe Phe Ala Val Ile			
	625	630	635	
30				
				2150
	TTC CTC GCC AGA TAC ACG GCC AAC CTG GGC GGC TTC ATG ATC CAA GAG Phe Leu Ala Arg Tyr Thr Ala Asn Leu Ala Ala Phe Met Ile Gln Glu			
	640	645	650	
35				
	CAA TAC ATC GAC ACT GTG TCG GGC CTC AGT GAC AAG AAG TTT CAG CGG			2198

	Gln Tyr Ile Asp Thr Val Ser Gly Leu Ser Asp Lys Lys Phe Gln Arg		
	655	660	665
			670
	SCT CAA GAT GAG TAC CCA CCT TTC CGC TTC GGC ACG GTG CCC AAC GGC		2246
5	Pro Gln Asp Gln Tyr Pro Pro Phe Arg Phe Gly Thr Val Pro Asn Gly		
	675	680	685
	AGC ACG GAG CGG AAC ATC CGC AGT AAC TAC CGT GAC ATG CAC ACC CAC		2294
	Ser Thr Glu Arg Asn Ile Arg Ser Asn Tyr Arg Asp Met His Thr His		
10	690	695	700
	ATG GTC AAG TTC AAC CAG CGC TCG GTG GAG GAC GCG CTC ACC AGC CTC		2342
	Met Val Lys Phe Asn Gln Arg Ser Val Glu Asp Ala Leu Thr Ser Leu		
	705	710	715
15	AAG ATG GGG AAG CTG GAT GCC TTC ATC TAT GAT GCT GTC CTC AAC		2390
	Lys Met Gly Lys Leu Asp Ala Phe Ile Tyr Asp Ala Ala Val Leu Asn		
	720	725	730
20	TAC ATG GCA GGC AAG GAC GAG GGC TGC AAG CTG GTC ACC ATT GGG TCT		2438
	Tyr Met Ala Gly Lys Asp Glu Gly Cys Lys Leu Val Thr Ile Gly Ser		
	735	740	745
	750	755	760
	GGC AAG GTC TTT GCT ACC ACT GGC TAC GGC ATC GCC ATG CAG AAG GAC		2486
25	Gly Lys Val Phe Ala Thr Thr Gly Tyr Gly Ile Ala Met Gln Lys Asp		
	765	770	775
	TCC CAC TGG AAG CGG GCC ATA GAC CTG GCG CTC TTG CAG TTC CTG GGG		2534
	Ser His Trp Lys Arg Ala Ile Asp Leu Ala Leu Leu Gln Phe Leu Gly		
30	780	785	790
	GAC GGA GAG ACA CAG AAA CTG GAG ACA GTG TGG CTC TCA GGG ATC TGC		2582
	Asp Gly Glu Thr Gln Lys Leu Glu Thr Val Trp Leu Ser Gly Ile Cys		
	795	800	805
35			

	CAG AAT GAG AAG AAC GAG GTG ATG AGC ACC AAG CTG GAC ATC GAC AAC		2630
	Gln Asn Glu Lys Asn Glu Val Met Ser Ser Lys Leu Asp Ile Asp Asn		
	800	805	810
5	ATG GCA GGC GTC TTC TAC ATG CTG CTG GTG GCC ATG GGG CTG GCC CTG		2678
	Met Ala Gly Val Phe Tyr Met Leu Leu Val Ala Met Gly Leu Ala Leu		
	815	820	825
	CTG GTC TTC GCG TGG GAG CAC CTG GTC TAC TGG AAG CTG CGC AAC TGG		2726
10	Leu Val Phe Ala Trp Glu His Leu Val Tyr Trp Lys Leu Arg His Ser		
	835	840	845
	GTG CCC AAC TCA TCC CAG CTG GAC TTC CTG CTG GCT TTC AGC AGG GGC		2774
	Val Pro Asn Ser Ser Gln Leu Asp Phe Leu Leu Ala Phe Ser Arg Gly		
15	850	855	860
	ATC TAC AGC TGC TTC AGC GGG GTG CAG AGC CTC GCC AGC CCA CGG CGG		2822
	Ile Tyr Ser Cys Phe Ser Gly Val Gln Ser Leu Ala Ser Pro Pro Arg		
	865	870	875
20	CAG GCC AGC CGG GAC CTC ACG GCC AGC TCG GCC CAG GCC AGC GTG CTC		2870
	Gln Ala Ser Pro Asp Leu Thr Ala Ser Ser Ala Gln Ala Ser Val Leu		
	880	885	890
25	AAG ATG CTG CAG GCA GGC CGC GAC ATG GTG ACC ACG CGG GGC GTA AGC		2918
	Lys Met Leu Gln Ala Ala Arg Asp Met Val Thr Thr Ala Gly Val Ser		
	895	900	905
	AGC TCC CTG GAC CGC GGC ACT CGC ACC ATC GAG AAT TGG GGT GGC GGC		2966
30	Ser Ser Leu Asp Arg Ala Thr Arg Thr Ile Glu Asn Trp Gly Gly Gly		
	915	920	925
	CGC CGT GCG CGG CCA CGG TCC CGG ACC CGG CGG TGT GGC CGG		3014
	Arg Arg Ala Pro Pro Pro Ser Pro Cys Pro Thr Pro Arg Ser Gly Pro		
35	930	935	940

	AGC CCA TGC CTG CCC ACC CCC GAC CCG CCC CCA GAG CCG AGC CCC ACG	3062		
	Ser Pro Cys Leu Pro Thr Pro Asp Pro Pro Pro Glu Pro Ser Pro Thr			
	945	950	955	
5				
	GGC TGG GGA CGG CCA GAC GGG GGT CGC CGG GCG CTT GTG CGC AGG GCT	3110		
	Gly Trp Gly Pro Pro Asp Gly Gly Arg Ala Ala Leu Val Arg Arg Ala			
	960	965	970	
10				
	CGG CAG CCC CGG GGC CGC CGG CGG ACG CGG GGG CGG CCC CTG TCC GAC	3158		
	Pro Gln Pro Pro Gly Arg Pro Pro Thr Pro Gly Pro Pro Leu Ser Asp			
	975	980	985	990
	GTC TCC CGA GTG TCG CGC CGC CCA GCC TGG GAG GCG CGG TGG CGG GTG	3206		
15	Val Ser Arg Val Ser Arg Arg Pro Ala Trp Glu Ala Arg Trp Pro Val			
	995	1000	1005	
	CGG ACC GGG CAC TGC GGG AGG CAC CTC TCG GCC TCC GAG CGG CCC CTG	3254		
	Arg Thr Gly His Cys Gly Arg His Leu Ser Ala Ser Glu Arg Pro Leu			
20	1010	1015	1020	
	TCG CCC GCG CGC TGT CAC TAC AGC TCC TTT CCT CGA GCG GAC CGA TCC	3302		
	Ser Pro Ala Arg Cys His Tyr Ser Ser Phe Pro Arg Ala Asp Arg Ser			
	1025	1030	1035	
25				
	GGC CGC CCC TTC CTC CGG CTC TTC CGG GAG CCC CGG GAG CTG GAG GAC	3350		
	Gly Arg Pro Phe Leu Pro Leu Phe Pro Glu Pro Pro Glu Leu Glu Asp			
	1040	1045	1050	
30				
	CTG CGG CTG CTC GGT CGG GAG CAG CTG GCG CGG CGG GAG GCG CTG CTG	3398		
	Leu Pro Leu Leu Gly Pro Glu Glu Leu Ala Arg Arg Glu Ala Leu Leu			
	1055	1060	1065	1070
	CAC CGG GCG TGG GCG CGG GGC TCG CGC CGG CGT CAC GCT TCC CTG CCC	3446		
35	His Ala Ala Trp Ala Arg Gly Ser Arg Pro Arg His Ala Ser Leu Pro			

	1075	1080	1085	
	AGC TCC GTG GCC GAG GCC TTC GCT CGG CCC AGC TCG CTG CCC GCT GGG			3494
	Ser Ser Val Ala Glu Ala Phe Ala Arg Pro Ser Ser Leu Pro Ala Gly			
5	1090	1095	1100	
	TGC ACC GGC CCC GCC TGC GCC CGC CCC GAC GGA CAC TCG GCC TGC AGG			3542
	Cys Thr Gly Pro Ala Cys Ala Arg Pro Asp Gly His Ser Ala Cys Arg			
	1105	1110	1115	
10				
	CGC TTG GCG CAG GCG CAG TCG ATG TGC TTG CGG ATC TAC CGG GAG GCC			3590
	Arg Leu Ala Gln Ala Gln Ser Met Cys Leu Pro Ile Tyr Arg Glu Ala			
	1120	1125	1130	
15				
	TGC CAG GAG GGC GAG CAG GCA GGG GCC CCC GGC TGG CAG CAC AGA CAG			3638
	Cys Gln Glu Gly Glu Gln Ala Gly Ala Pro Ala Trp Gln His Arg Gln			
	1135	1140	1145	1150
	CAC GTC TGC CTG CAC GCC CAC CAC CTG CCA TTT TGC TGG GGG GCT			3686
20	His Val Cys Leu His Ala His Ala His Leu Pro Phe Cys Trp Gly Ala			
	1155	1160	1165	
	GTC TGT CCT CAC CTT CCA CCC TGT GCC AGC CAC GGC TCC TGG CTC TCC			3734
	Val Cys Pro His Leu Pro Pro Cys Ala Ser His Gly Ser Trp Leu Ser			
25	1170	1175	1180	
	GGG GCC TGG GGG CCT CTG GGG CAC AGG GGC AAG ACT CTG GGG CTG GGC			3782
	Gly Ala Trp Gly Pro Leu Gly His Arg Gly Arg Thr Leu Gly Leu Gly			
	1185	1190	1195	
30				
	ACA GGC TAC AGA GAC AGT GGG GGA CTG GAC GAG ATC AGC AGG GTA GCC			3830
	Thr Gly Tyr Arg Asp Ser Gly Gly Leu Asp Glu Ile Ser Arg Val Ala			
	1200	1205	1210	
35				
	CGT GGG ACG CAA GGC TTC CGG GGA CCC TGC ACG TGG AGA CGG ATC TCC			3878

	Arg Gly Thr Gin Gly Phe Pro Gly Pro Cys Thr Trp Arg Arg Ile Ser				
	1216	1220	1225	1231	
	AGT CTG GAG TCA GAA GTG TGAGTTATCA GGCACTCAGG CTCCGAGCCA				3926
5	Ser Leu Glu Ser Glu Val				
	1235				
	GCTGGATTCT CTGCCTGCGA CTGTCAAGGT TAAGCGGCAG GCAGGATTGG GCTTTCTGG				3986
10	CTTCTACCAT GAAATCTGG CCATGGGACC CCAGTGACAG ATGATGTCTT CCATGGTCAT				4346
	CAGTGACCTC AGTAGCCTCA AATCATGGTG AGGGCTGGC TTTTGCTGTC CTCTTCTCAC				4106
	GCAGAGTTCT GCCAGGAGGG TGTGCTGTGG GGGTCAGACT CCTGAGGCTC TCCCTTCCCT				4166
15	GGGGCTAGCC AGTTACTGGT CATGCCTGCT GTGGGCATGG AGGCTGGAAC TTGTGGTTGA				4226
	GGCAGGGCCA TCCCAGATCCT TGCTCTACCT GGCTAGAGTT CCTTCTCATC AGAGCACTGG				4286
20	GACATTAAAC CCACCTTTTC CCAGAAAAAA AAAAAAAAAA AAAAAAAAAA AAAG				4340

(2) INFORMATION FOR SEQ ID NO:6:

25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1236 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Gly Gly Ala Leu Gly Pro Ala Leu Leu Leu Thr Ser Leu Phe Gly

35 1 5 10 15

Ala Trp Ala Gly Leu Gly Pro Gly Gln Gly Glu Gln Gly Met Thr Val
 20 25 30

5 Ala Val Val Phe Ser Ser Ser Gly Pro Pro Gln Ala Gln Phe Arg Val
 35 40 45

Arg Leu Thr Pro Gln Ser Phe Leu Asp Leu Pro Leu Glu Ile Gln Pro
 50 55 60

10 Leu Thr Val Gly Val Asn Thr Thr Asn Pro Ser Ser Leu Leu Thr Gln
 65 70 75 80

Ile Cys Gly Leu Leu Gly Ala Ala His Val His Gly Ile Val Phe Glu
15 85 90 95

Asp Asn Val Asp Thr Glu Ala Val Ala Gln Ile Leu Asp Phe Ile Ser
 100 105 110

20 Ser Gln Thr His Val Pro Ile Leu Ser Ile Ser Gly Gly Ser Ala Val
 115 120 125

Val Leu Thr Pro Lys Glu Pro Gly Ser Ala Phe Leu Gln Leu Gly Val
 130 135 140

25
 Ser Leu Glu Gln Gln Leu Gln Val Leu Phe Lys Val Leu Glu Glu Tyr
 145 150 155 160

Asp Trp Ser Ala Phe Ala Val Ile Thr Ser Leu His Pro Gly His Ala
30 165 170 175

Leu Phe Leu Glu Gly Val Arg Ala Val Ala Asp Ala Ser His Val Ser
 180 185 190

35 Trp Arg Leu Leu Asp Val Val Thr Leu Glu Leu Asp Pro Gly Gly Pro

195 200 205
Arg Ala Arg Thr Gln Arg Leu Leu Arg Gln Leu Asp Ala Pro Val Phe
210 215 220
5
Val Ala Tyr Cys Ser Arg Glu Glu Ala Glu Val Leu Phe Ala Glu Ala
225 230 235 240

Ala Gln Ala Gly Leu Val Gly Pro Gly His Val Trp Leu Val Pro Asn
10 245 250 255

Leu Ala Leu Gly Ser Thr Asp Ala Pro Pro Ala Thr Phe Pro Val Gly
260 265 270

15 Leu Ile Ser Val Val Thr Glu Ser Trp Arg Leu Ser Leu Arg Gln Lys
275 280 285

Val Arg Asp Gly Val Ala Ile Leu Ala Leu Gly Ala His Ser Tyr Trp
290 295 300
20
Arg Gln His Gly Thr Leu Pro Ala Pro Ala Gly Asp Cys Arg Val His
305 310 315 320

Pro Gly Pro Val Ser Pro Ala Arg Glu Ala Phe Tyr Arg His Leu Leu
25 325 330 335

Asn Val Thr Trp Glu Gly Arg Asp Phe Ser Phe Ser Pro Gly Gly Tyr
340 345 350

30 Leu Val Gin Pro Thr Met Val Val Ile Ala Leu Asn Arg His Arg Leu
355 360 365

Trp Glu Met Val Gly Arg Trp Glu His Gly Val Leu Tyr Met Lys Tyr
370 375 380
35

Pro Val Trp Pro Arg Tyr Ser Ala Ser Leu Gln Pro Val Val Asp Ser
 385 390 395 400

 Arg His Leu Thr Val Ala Thr Leu Glu Glu Arg Pro Phe Val Ile Val
5 405 410 415

 Glu Ser Pro Asp Pro Gly Thr Gly Gly Cys Val Pro Asn Thr Val Pro
 420 425 430

10 Cys Arg Arg Gln Ser Asn His Thr Phe Ser Ser Gly Asp Val Ala Pro
 435 440 445

 Tyr Thr Lys Leu Cys Cys Lys Gly Phe Cys Ile Asp Ile Leu Lys Lys
 450 455 460
15
 Leu Ala Arg Val Val Lys Phe Ser Tyr Asp Leu Tyr Leu Val Thr Asn
 465 470 475 480

 Gly Lys His Gly Lys Arg Val Arg Gly Val Trp Asn Gly Met Ile Gly
20 485 490 495

 Glu Val Tyr Tyr Lys Arg Ala Asp Met Ala Ile Gly Ser Leu Thr Ile
 500 505 510

25 Asn Glu Glu Arg Ser Glu Ile Val Asp Phe Ser Val Pro Phe Val Glu
 515 520 525

 Thr Gly Ile Ser Val Met Val Ala Arg Ser Asn Gly Thr Val Ser Pro
 530 535 540
30
 Ser Ala Phe Leu Glu Pro Tyr Ser Pro Ala Val Trp Val Met Met Phe
 545 550 555 560

 Val Met Cys Leu Thr Val Val Ala Ile Thr Val Phe Met Phe Glu Tyr
35 565 570 575

Phe Ser Pro Val Ser Tyr Asn Gln Asn Leu Thr Arg Gly Lys Lys Ser
 580 585 590

5 Gly Gly Pro Ala Phe Thr Ile Gly Lys Ser Val Trp Leu Leu Trp Ala
 595 600 605

Leu Val Phe Asn Asn Ser Val Pro Ile Glu Asn Pro Arg Gly Thr Thr
 610 615 620

10 Ser Lys Ile Met Val Leu Val Trp Ala Phe Phe Ala Val Ile Phe Leu
 625 630 635 640

Ala Arg Tyr Thr Ala Asn Leu Ala Ala Phe Met Ile Gln Glu Gln Tyr
 15 645 650 655

Ile Asp Thr Val Ser Gly Leu Ser Asp Lys Lys Phe Gln Arg Pro Gln
 660 665 670

20 Asp Gln Tyr Pro Pro Phe Arg Phe Gly Thr Val Pro Asn Gly Ser Thr
 675 680 685

Glu Arg Asn Ile Arg Ser Asn Tyr Arg Asp Met His Thr His Met Val
 690 695 700

25 Lys Phe Asn Gln Arg Ser Val Glu Asp Ala Leu Thr Ser Leu Lys Met
 705 710 715 720

Gly Lys Leu Asp Ala Phe Ile Tyr Asp Ala Ala Val Leu Asn Tyr Met
 725 730 735

30 Ala Gly Lys Asp Glu Gly Cys Lys Leu Val Thr Ile Gly Ser Gly Lys
 740 745 750

35 Val Phe Ala Thr Thr Gly Tyr Gly Ile Ala Met Gln Lys Asp Ser His

755

760

765

Trp Lys Arg Ala Ile Asp Leu Ala Leu Leu Gin Phe Leu Gly Asp Gly

770

775

780

5

Glu Thr Gln Lys Leu Glu Thr Val Trp Leu Ser Gly Ile Cys Gln Asn

785

790

795

800

Glu Lys Asn Glu Val Met Ser Ser Lys Leu Asp Ile Asp Asn Met Ala

10

805

810

815

Gly Val Phe Tyr Met Leu Leu Val Ala Met Gly Leu Ala Leu Leu Val

820

825

830

15

Phe Ala Trp Glu His Leu Val Tyr Trp Lys Leu Arg His Ser Val Pro

835

840

845

Asn Ser Ser Gln Leu Asp Phe Leu Leu Ala Phe Ser Arg Gly Ile Tyr

850

855

860

20

Ser Cys Phe Ser Gly Val Gln Ser Leu Ala Ser Pro Pro Arg Gln Ala

865

870

875

880

Ser Pro Asp Leu Thr Ala Ser Ser Ala Gln Ala Ser Val Leu Lys Met

25

885

890

895

Leu Gln Ala Ala Arg Asp Met Val Thr Thr Ala Gly Val Ser Ser Ser

900

905

910

30

Leu Asp Arg Ala Thr Arg Thr Ile Glu Asn Trp Gly Gly Arg Arg

915

920

925

Ala Pro Pro Pro Ser Pro Cys Pro Thr Pro Arg Ser Gly Pro Ser Pro

930

935

940

35

Cys Leu Pro Thr Pro Asp Pro Pro Glu Pro Ser Pro Thr Gly Trp
 945 950 955 960

5 965 970 975
 Gly Pro Pro Asp Gly Gly Arg Ala Ala Leu Val Arg Arg Ala Pro Gln

Pro Pro Gly Arg Pro Pro Thr Pro Gly Pro Pro Leu Ser Asp Val Ser
 980 985 990

10 995 1000 1005
 Arg Val Ser Arg Arg Pro Ala Trp Glu Ala Arg Trp Pro Val Arg Thr

Gly His Cys Gly Arg His Leu Ser Ala Ser Glu Arg Pro Leu Ser Pro
 1010 1015 1020

15 1025 1030 1035 1040
 Ala Arg Cys His Tyr Ser Ser Phe Pro Arg Ala Asp Arg Ser Gly Arg

20 1045 1050 1055
 Pro Phe Leu Pro Leu Phe Pro Glu Pro Pro Glu Leu Glu Asp Leu Pro

Leu Leu Gly Pro Glu Gln Leu Ala Arg Arg Glu Ala Leu Leu His Ala
 1060 1065 1070

25 1075 1080 1085
 Ala Trp Ala Arg Gly Ser Arg Pro Arg His Ala Ser Leu Pro Ser Ser

Val Ala Glu Ala Phe Ala Arg Pro Ser Ser Leu Pro Ala Gly Cys Thr
 1090 1095 1100

30 1105 1110 1115 1120
 Gly Pro Ala Cys Ala Arg Pro Asp Gly His Ser Ala Cys Arg Arg Leu

35 1125 1130 1135
 Ala Gln Ala Gln Ser Met Cys Leu Pro Ile Tyr Arg Glu Ala Cys Gln

Glu Gly Glu Gln Ala Gly Ala Pro Ala Trp Gln His Arg Gln His Val

1140 1145 1150

5 Cys Leu His Ala His Ala His Leu Pro Phe Cys Trp Gly Ala Val Cys

1155 1160 1165

Pro His Leu Pro Pro Cys Ala Ser His Gly Ser Trp Leu Ser Gly Ala

1170 1175 1180

10

Trp Gly Pro Leu Gly His Arg Gly Arg Thr Leu Gly Leu Gly Thr Gly

1185 1190 1195 1200

Tyr Arg Asp Ser Gly Gly Leu Asp Glu Ile Ser Arg Val Ala Arg Gly

15 1205 1210 1215

Thr Gln Gly Phe Pro Gly Pro Cys Thr Trp Arg Arg Ile Ser Ser Leu

1220 1225 1230

20 Glu Ser Glu Val

1235

(2) INFORMATION FOR SEQ ID NO:7:

25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: both

30 (D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

35 (A) NAME KEY: CDS

(B) LOCATION: 2..22

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

5 C TCT GAG GCT CAG CCT GTC CCC AG
Ser Glu Ala Gln Pro Val Pro
1 5

24

10 (2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

20 Ser Glu Ala Gln Pro Val Pro
1 5

25 (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGAAGGGGCT G

11

5 (2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4808 base pairs
- (B) TYPE: nucleic acid
- 10 (C) STRANDEDNESS: both
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

15 (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 311..4705

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

20

ATCATGGGAC CGGGTGAGCG CTGAGAACATCG CGGCCGCGAGC CATCAGCCCT GGAGATGACC 60

AGGAGCGGCC ACTGCTGAGA ACTATGTGGA GAGAGGCTGC GAGCCCTGCT GCAGAGCCTC 120

25

CGGCTGGGAT AGCAGCCCCC CGTGGGGCG ATGCAGACAG CGCAGGGACAG CCAGGGGAGC 180

GGGCTGGGGC CGCACCATGC CGGAAACCGGC TAAACCCGGT GGCTGCTGAG GCGGCCGAGA 240

TGGCTGGTGGC CGCAGCGCGC CCCACTGCAT CCTCGACCTT CCTGGGCTAC AGGGACCGTC 300

30

AGTGGGCACT ATG GGE AGA GTG GGC TAT TGG ACC CTG CTG GTG CTG CCG 343

Met Gly Arg Val Gly Tyr Trp Thr Leu Leu Val Leu Pro

1

5

10

35

GCC CTT CTG GTC TGG CGC GGT CCG GCG CCG AGC GCG GCG GCG GAG AAG 397

Ala Leu Leu Val Trp Arg Gly Pro Ala Pro Ser Ala Ala Ala Glu Lys
 15 20 25

GGT CCC CCC GCG CTA AAT ATT GCG GTG ATG CTG GGT AAC AGC CAC GAC 445
 5 Gly Pro Pro Ala Leu Asn Ile Ala Val Met Leu Gly His Ser His Asp
 30 35 40 45

GTG ACA GAG CGC GAA CTT CGA ACA CTG TGG GGC CCC GAG CAG GCG GCG 493
 Val Thr Glu Arg Glu Leu Arg Thr Leu Trp Gly Pro Glu Gln Ala Ala
 10 50 55 60

GGG CTG CCC CTG GAC GTG AAC GTG GTA GCT CTG CTG ATG AAC CGC ACC 541
 Gly Leu Pro Leu Asp Val Asn Val Val Ala Leu Leu Met Asn Arg Thr
 65 70 75

15 GAC CCC AAG AAG CTC ATC ACG CAC GTG TGC GAC CTC ATG TCC GGG GCA 589
 Asp Pro Lys Ser Leu Ile Thr His Val Cys Asp Leu Met Ser Gly Ala
 80 85 90

20 CGC ATC CAC GGC CTC GTG TTT GGG GAC GAC ACG CAG GAG GCC GTA 637
 Arg Ile His Gly Leu Val Phe Gly Asp Asp Thr Asp Gln Glu Ala Val
 95 100 105

GCC CAG ATG CTG GAT TTT ATC TCC TCC CAC ACC TTC GTC CCC ATC TTG 685
 25 Ala Gln Met Leu Asp Phe Ile Ser Ser His Thr Phe Val Pro Ile Leu
 110 115 120 125

GGC ATT CAT GGG GGC GCA TCT ATC ATG ATG GCT GAC AAG GAT CCG ACG 733
 Gly Ile His Gly Gly Ala Ser Met Ile Met Ala Asp Lys Asp Pro Thr
 30 130 135 140

TCT ACC TTC TPC CAG TTT GGA GCG TCC ATC CAG CAG CAA GCC ACG GTC 781
 Ser Thr Phe Phe Gln Phe Gly Ala Ser Ile Gln Gln Ala Thr Val
 145 150 155

35

	ATG CTG AAG ATC ATG CAG GAT TAT GAC TGG CAT GTC TTC TCC CTG GTG		623	
	Met Leu Lys Ile Met Gln Asp Tyr Asp Trp His Val Phe Ser Leu Val			
	160	165	170	
5	ACC ACT ATC TTC CCT GGC TAC AGG GAA TTC ATC AGC TTC GTC AAG ACC		677	
	Thr Thr Ile Phe Pro Gly Tyr Arg Glu Phe Ile Ser Phe Val Lys Thr			
	175	180	185	
	ACA CTG GAC AAC AGC TTT GTG GGC TGG GAC ATG CAG AAT GTG ATC ACA		925	
10	Thr Val Asp Asn Ser Phe Val Gly Trp Asp Met Gln Asn Val Ile Thr			
	190	195	200	205
	CTG GAC ACT TCC TTT GAG GAT GCA AAG ACA CAA GTC CAG CTG AAG AAG		973	
	Leu Asp Thr Ser Phe Glu Asp Ala Lys Thr Gln Val Gln Leu Lys Lys			
15	210	215	220	
	ATC CAC TCT TCT GTC ATC TTG CTC TAC TGT TCC AAA GAC GAG GCT GTT		1021	
	Ile His Ser Ser Val Ile Leu Leu Tyr Cys Ser Lys Asp Glu Ala Val			
	225	230	235	
20	CTC ATT CTG AGT GAG GCC CGC TCC CTT GGC CTC ACC GGG TAT GAT TTC		1069	
	Leu Ile Leu Ser Glu Ala Arg Ser Leu Gly Leu Thr Gly Tyr Asp Phe			
	240	245	250	
25	TTC TGG ATT GTC CCC AGC TTG GTC TCT GGG AAC ACG GAG CTC ATC CCA		1117	
	Phe Trp Ile Val Pro Ser Leu Val Ser Gly Asn Thr Glu Leu Ile Pro			
	255	260	265	
	AAA GAG TTT CCA TCG GGA CTC ATT TCT GTC TCC TAC GAT GAC TGG GAC		1165	
30	Lys Glu Phe Pro Ser Gly Leu Ile Ser Val Ser Tyr Asp Asp Trp Asp			
	270	275	280	285
	TAC AGC CTG GAG GCG AGA GTG AGG GAC GGC ATT GGC ATC CTA ACC ACC		1213	
	Tyr Ser Leu Glu Ala Arg Val Arg Asp Gly Ile Gly Ile Leu Thr Thr			
35	290	295	300	

	GCT GCA TCT TCT ATG CTG GAG AAG TTC TCC TAC ATC CCC GAG GCC AAG		1261	
	Ala Ala Ser Ser Met Leu Glu Lys Phe Ser Tyr Ile Pro Glu Ala Lys			
	305	310	315	
5				
	GCC AGC TGC TAC GGG CAG ATG GAG AGG CCA GAG GTC CGG ATG CAC ACC		1309	
	Ala Ser Cys Tyr Gly Gln Met Glu Arg Pro Glu Val Pro Met His Thr			
	320	325	330	
10	TTG CAC CCA TTT ATG GTC AAT GTT ACA TGG GAT GGC AAA GAC TTA TCC		1357	
	Leu His Pro Phe Met Val Asn Val Thr Trp Asp Gly Lys Asp Leu Ser			
	335	340	345	
	TTC ACT GAG GAA GGC TAC CAG GTG CAC CCC AGG CTG GTG GTG ATT GTG		1405	
15	Phe Thr Glu Glu Gly Tyr Gln Val His Pro Arg Leu Val Val Ile Val			
	350	355	360	365
	CTG AAC AAA GAC CGG GAA TGG GAA AAG GTG GGC AAG TGG GAG AAC CAT		1453	
	Leu Asn Lys Asp Arg Glu Trp Glu Lys Val Gly Lys Trp Glu Asn His			
20	370	375	380	
	ACG CTG AGC CTG AGG CAC GCC GTG TGG CCC AGG TAC AAG TCC TTC TCC		1501	
	Thr Leu Ser Leu Arg His Ala Val Trp Pro Arg Tyr Lys Ser Phe Ser			
	385	390	395	
25				
	GAC TGT GAG CGG GAT GAC AAC CAT CTC AGC ATC GTC ACC CTG GAG GAG		1549	
	Asp Cys Glu Pro Asp Asp Asn His Leu Ser Ile Val Thr Leu Glu Glu			
	400	405	410	
30	GCC CCA TTC GTC ATC GTG GAA GAC ATA GAC CCC CTG ACC GAG ACG TGT		1597	
	Ala Pro Phe Val Ile Val Glu Asp Ile Asp Pro Leu Thr Glu Thr Cys			
	415	420	425	
	GTG AGG AAC ACC GTG CCA TGT CGG AAG TTC GTC AAA ATC AAC AAT TCA		1645	
35	Val Arg Asn Thr Val Pro Cys Arg Lys Phe Val Lys Ile Asn Asn Ser			

	430	435	440	445	
	ACC AAT GAG GGG ATG AAT GTG AAG AAA TGC TGC AAG GGG TTC TGC ATT				1693
	Thr Asn Glu Gly Met Asn Val Lys Lys Cys Cys Lys Gly Phe Cys Ile				
5	450	455	460		
	GAT ATT CTG AAG AAG CTT TCC AGA ACT GTG AAG TTT ACT TAC GAC CTC				1741
	Asp Ile Leu Lys Lys Leu Ser Arg Thr Val Lys Phe Thr Tyr Asp Leu				
	465	470	475		
10					
	TAT CTG GTG ACC AAT GGG AAG CAT GGC AAG AAA GTT AAC AAT GTG TGG				1789
	Tyr Leu Val Thr Asn Gly Lys His Gly Lys Lys Val Asn Asn Val Trp				
	480	485	490		
15	AAT GGA ATG ATC GGT GAA GTG GTC TAT CAA CGG GCA GTC ATG GCA GTT				1837
	Asn Gly Met Ile Gly Glu Val Val Tyr Gln Arg Ala Val Met Ala Val				
	495	500	505		
	GGC TCG CTC ACC ATC AAT GAG GAA CGT TCT GAA GTG GTG GAC TTC TCT				1885
20	Gly Ser Leu Thr Ile Asn Glu Glu Arg Ser Glu Val Val Asp Phe Ser				
	510	515	520	525	
	GTG CCC TTT GTG GAA ACG GGA ATC AGT GTC ATG GTT TCA AGA AGT AAT				1933
	Val Pro Phe Val Glu Thr Gly Ile Ser Val Met Val Ser Arg Ser Asn				
25	530	535	540		
	GGC ACC GTC TCA CCT TCT GCT TTT CTA GAA CCA TTC AGC GCC TCT GTC				1981
	Gly Thr Val Ser Pro Ser Ala Phe Leu Glu Pro Phe Ser Ala Ser Val				
	545	550	555		
30					
	TGG GTG ATG ATG TTT GTG ATG GTG CTC ATT GTT TCT GCC ATA GCT GTT				2029
	Trp Val Met Met Phe Val Met Leu Leu Ile Val Ser Ala Ile Ala Val				
	560	565	570		
35	TGG GTC TTG GAT TAC TCC AGC CCT GTT GGA TAC AAC AGA AAC TTA GCC				2077

	Trp Val Leu Asp Tyr Ser Ser Pro Val Gly Tyr Asn Arg Asn Leu Ala		
	575	580	585
5	AAA GGG AAA GCA CCC CAT GGG CCT TCT TTT ACA ATT GGA AAA GCT ATA	2125	
	Lys Gly Lys Ala Pro His Gly Pro Ser Phe Thr Ile Gly Lys Ala Ile		
	590	595	600
	TGG CTT CTT TGG GGC CTG GTG TTC AAT AAC TCC GTG CCT GTC CAG AAT	2173	
	Trp Leu Leu Trp Gly Leu Val Phe Asn Asn Ser Val Pro Val Gln Asn		
10	610	615	620
	CCT AAA GGG ACC ACC AGC AAG ATC ATG GTA TCT GTA TGG GCC TTC TTC	2221	
	Pro Lys Gly Thr Thr Ser Lys Ile Met Val Ser Val Trp Ala Phe Phe		
	625	630	635
15			
	GCT GTC ATA TTC CTG GCT AGC TAC ACA GCC AAT CTG GCT GCC TTC ATG	2269	
	Ala Val Ile Phe Leu Ala Ser Tyr Thr Ala Asn Leu Ala Phe Met		
	640	645	650
20	ATC CAA GAG GAA TTT GTG GAC CAA GTG ACC GGC CTC AGT GAC AAA AAG	2317	
	Ile Gln Glu Glu Phe Val Asp Gln Val Thr Gly Leu Ser Asp Lys Lys		
	655	660	665
25	TTT CAG AGA CCT CAT GAC TAT TCC CCA CCT TTT CGA TTT GGG ACA GTG	2365	
	Phe Gln Arg Pro His Asp Tyr Ser Pro Pro Phe Arg Phe Gly Thr Val		
	670	675	680
30	CCT AAT GGA AGC ACC GAG AGA AAC ATT CGG AAT AAC TAT CCC TAC ATG	2413	
	Pro Asn Gly Ser Thr Glu Arg Asn Ile Arg Asn Asn Tyr Pro Tyr Met		
	690	695	700
35	CAT CAG TAC ATG ACC AAA TTT AAT CAG AAA GGA GTA GAG GAC GGC TTG	2461	
	His Gln Tyr Met Thr Lys Phe Asn Gln Lys Gly Val Glu Asp Ala Leu		
	705	710	715

	GTC AGC CTG AAA ACG CGG AAG CTG GAC GCT TTC ATC TAC GAT GCC GCA		2519
	Val Ser Leu Lys Thr Gly Lys Leu Asp Ala Phe Ile Tyr Asp Ala Ala		
	720	725	730
5	GTC TTG AAT TAC AAG GCT GGG AGG GAT GAA GGC TGC AAG CTG GTG ACC		2557
	Val Leu Asn Tyr Lys Ala Gly Arg Asp Glu Gly Cys Lys Leu Val Thr		
	735	740	745
	ATC GGG AGT GGG TAC ATC TTT GCC ACC ACC GGT TAT GGA ATT GCC CTT		2605
10	Ile Gly Ser Gly Tyr Ile Phe Ala Thr Thr Gly Tyr Gly Ile Ala Leu		
	750	755	760
	765		
	CAG AAA GGC TCT CCT TGG AAG AGG CAG ATC GAC CTG GCC TTG CTT CAG		2653
	Gln Lys Gly Ser Pro Trp Lys Arg Gln Ile Asp Leu Ala Leu Leu Gln		
15	770	775	780
	TTT GTG GGT GAT GGT GAG ATG GAG GAG CTG GAG ACC CTG TGG CTC ACT		2701
	Phe Val Gly Asp Gly Glu Met Glu Glu Leu Glu Thr Leu Trp Leu Thr		
	785	790	795
20	GGG ATC TGC CAC AAC GAG AAG AAC GAG GTC ATG AGC AGC CAG CTG GAC		2749
	Gly Ile Cys His Asn Glu Lys Asn Glu Val Met Ser Ser Gln Leu Asp		
	800	805	810
25	ATT GAC AAC ATG GCG GGC GTA TTC TAC ATG CTG GCT GCC GCC ATG GCC		2797
	Ile Asp Asn Met Ala Gly Val Phe Tyr Met Leu Ala Ala Ala Met Ala		
	815	820	825
	CTT AGC CTC ATC ACC TTC ATC TGG GAG GAC CTC TTC TAC TGG AAG CTG		2845
30	Leu Ser Leu Ile Thr Phe Ile Trp Glu His Leu Phe Tyr Trp Lys Leu		
	830	835	840
	845		
	CGG TTC TGT TTC ACG GGC GTG TGC TCC GAC CGG CCT GGG TTG CTC TTC		2893
	Arg Phe Cys Phe Thr Gly Val Cys Ser Asp Arg Pro Gly Leu Leu Phe		
35	850	855	860

	TCC ATC AGC AGG GGC ATC TAC AGC TGC ATT CAT GGA GTG CAC ATT GAA	2341
	Ser Ile Ser Arg Gly Ile Tyr Ser Cys Ile His Gly Val His Ile Glu	
	865 870 875	
5		
	GAA AAG AAG AAG TCT CCA GAC TTC AAT CTG ACG GGA TCC CAG AGC AAC	2989
	Glu Lys Lys Lys Ser Pro Asp Phe Asn Leu Thr Gly Ser Gln Ser Asn	
	880 885 890	
10	ATG TTA AAA CTC CTC CGG TCA GCC AAA AAC ATT TCC AGC ATG TCC AAC	3037
	Met Leu Lys Leu Leu Arg Ser Ala Lys Asn Ile Ser Ser Met Ser Asn	
	895 900 905	
	ATG AAC TCC TCA AGA ATG GAC TCA CCC AAA AGA GCT GCT GAC TTC ATC	3085
15	Met Asn Ser Ser Arg Met Asp Ser Pro Lys Arg Ala Ala Asp Phe Ile	
	910 915 920 925	
	CAA AGA GGT TCC CTC ATC ATG GAC ATG GTT TCA GAT AAG GGG AAT TTG	3133
	Gln Arg Gly Ser Leu Ile Met Asp Met Val Ser Asp Lys Gly Asn Leu	
20	930 935 940	
	ATG TAC TCA GAC AAC AGG TCC TTT CAG GGG AAA GAG AGC ATT TTT GGA	3181
	Met Tyr Ser Asp Asn Arg Ser Phe Gln Gly Lys Glu Ser Ile Phe Gly	
	945 950 955	
25		
	GAC AAC ATG AAC GAA CTC CAA ACA TTT GTG GCC AAC CGG CAG AAG GAT	3229
	Asp Asn Met Asn Glu Leu Gln Thr Phe Val Ala Asn Arg Gln Lys Asp	
	960 965 970	
30	AAC CTC AAT AAC TAT GTA TTC CAG GGA CAA CAT CCT CTT ACT CTC AAT	3277
	Asn Leu Asn Asn Tyr Val Phe Gln Gly Gln His Pro Leu Thr Leu Asn	
	975 980 985	
35	GAG TCC AAC CCT AAC ACG GTG GAG GTG GCC GTG AGC ACA GAA TCC AAA	3325
	Glu Ser Asn Pro Asn Thr Val Glu Val Ala Val Ser Thr Glu Ser Lys	

	990	995	1000	1005	
	GCG AAC TCT AGA CCC CGG CAG CTG TGG AAG AAA TCC GTG GAT TCC ATA				3373
	Ala Asn Ser Arg Pro Arg Gln Leu Trp Lys Lys Ser Val Asp Ser Ile				
5	1010	1015	1020		
	CGC CAG GAT TCA CTA TCC CAG AAT CCA GTC TCC CAG AGG GAT GAG GCA				3421
	Arg Gln Asp Ser Leu Ser Gln Asn Pro Val Ser Gln Arg Asp Glu Ala				
	1025	1030	1035		
10					
	ACA GCA GAG AAT AGG ACC CAC TCC CTA AAG AGC CCT AGG TAT CTT CCA				3469
	Thr Ala Glu Asn Arg Thr His Ser Leu Lys Ser Pro Arg Tyr Leu Pro				
	1040	1045	1050		
15	GAA GAG ATG GCC CAC TCT GAC ATT TCA GAA ACG TCA AAT CGG GCC ACG				3517
	Glu Glu Met Ala His Ser Asp Ile Ser Glu Thr Ser Asn Arg Ala Thr				
	1055	1060	1065		
	TGC CAC AGG GAA CCT GAC AAC AGT AAG AAC CAC AAA ACC AAG GAC AAC				3565
20	Cys His Arg Glu Pro Asp Asn Ser Lys Asn His Lys Thr Lys Asp Asn				
	1070	1075	1080	1085	
	TTT AAA AGG TCA GTG GCC TCC AAA TAC CCC AAG GAC TGT AGT GAG GTC				3613
	Phe Lys Arg Ser Val Ala Ser Lys Tyr Pro Lys Asp Cys Ser Glu Val				
25	1090	1095	1100		
	GAG CGC ACC TAC CTG AAA ACC AAA TCA AGC TCC CCT AGA GAC AAG ATC				3661
	Glu Arg Thr Tyr Leu Lys Thr Lys Ser Ser Ser Pro Arg Asp Lys Ile				
	1105	1110	1115		
30					
	TAC ACT ATA GAT GGT GAG AAG GAG CCT GGT TTC CAC TTA GAT CCA CCC				3709
	Tyr Thr Ile Asp Gly Glu Lys Glu Pro Gly Phe His Leu Asp Pro Pro				
	1120	1125	1130		
35	CAG TTT GTT GAA AAT GTG ACC CTG CTC GAG AAC GTG GAC TTC CCG GAC				3757

	Gln Phe Val Glu Asn Val Thr Leu Pro Glu Asn Val Asp Phe Pro Asp		
	1135	1140	1145
5	CCC TAC CAG GAT CCC AGT GAA AAC TTC CGC AAG GGG GAC TCC ACG CTG	3805	
	Pro Tyr Gln Asp Pro Ser Glu Asn Phe Arg Lys Gly Asp Ser Thr Leu		
	1150	1155	1160
10	CCA ATG AAC CGG AAC CCC TTG CAT AAT GAA GAG GGG CTT TCC AAC AAC	3853	
	Pro Met Asn Arg Asn Pro Leu His Asn Glu Glu Gly Leu Ser Asn Asn		
	1170	1175	1180
15	GAC CAG TAT AAA CTC TAC TCC AAG CAC TTC ACC TTG AAA GAC AAG GGT	3901	
	Asp Gln Tyr Lys Leu Tyr Ser Lys His Phe Thr Leu Lys Asp Lys Gly		
	1185	1190	1195
20	TCC CCG CAC AGT GAG ACC AGC GAG CGA TAC CGG CAG AAC TCC ACG CAC	3949	
	Ser Pro His Ser Glu Thr Ser Glu Arg Tyr Arg Gln Asn Ser Thr His		
	1200	1205	1210
25	TGC AGA AGC TGC CTT TCC AAC ATG CCC ACC TAT TCA GGC CAC TTC ACC	3997	
	Cys Arg Ser Cys Leu Ser Asn Met Pro Thr Tyr Ser Gly His Phe Thr		
	1215	1220	1225
30	ATG AGG TCC CCC TTC AAG TGC GAT GCC TGC CTG CGG ATG GGG AAC CTC	4045	
	Met Arg Ser Pro Phe Lys Cys Asp Ala Cys Leu Arg Met Gly Asn Leu		
	1230	1235	1240
35	TAT GAC ATC GAT GAA GAC CAG ATG CTT CAG GAG ACA GGT AAC CCA GCC	4093	
	Tyr Asp Ile Asp Gln Asp Gln Met Leu Gln Glu Thr Gly Asn Pro Ala		
	1250	1255	1260
	ACC GGG GAG CAG GTC TAC CAG CAG GAC TGG GCA CAG AAC AAT GCG CTT	4141	
	Thr Gly Gln Gln Val Tyr Gln Gln Asp Trp Ala Gln Asn Asn Ala Leu		
	1265	1270	1275

	CAA TTA CAA AAG AAC AAG CTA AGG ATT AGC CGT CAG CAT TCC TAC GAT		4169	
	Gln Leu Gln Lys Asn Lys Leu Arg Ile Ser Arg Gln His Ser Tyr Asp			
	1280	1285	1290	
5	AAC ATT GTC GAC AAA CCT AGG GAG CTA GAC CTT AGC AGG CCC TCC CGG		4237	
	Asn Ile Val Asp Lys Pro Arg Glu Leu Asp Leu Ser Arg Pro Ser Arg			
	1295	1300	1305	
10	AGC ATA AGC CTC AAG GAC AGG GAA CGG CTT CTG GAG GGA AAT TTT TAC		4285	
	Ser Ile Ser Leu Lys Asp Arg Glu Arg Leu Leu Glu Gly Asn Phe Tyr			
	1310	1315	1320	1325
15	GGC AGC CTG TTT AGT GTC CCC TCA AGC AAA CTC TCG GGG AAA AAA AGC		4323	
	Gly Ser Leu Phe Ser Val Pro Ser Ser Lys Leu Ser Gly Lys Lys Ser			
	1330	1335	1340	
20	TCC CTT TTC CCC CAA GGT CTG GAG GAC AGC AAG AGG AGC AAG TCT CTC		4381	
	Ser Leu Phe Pro Gln Gly Leu Glu Asp Ser Lys Arg Ser Lys Ser Leu			
	1345	1350	1355	
25	TTG CCA GAC CAC ACC TCC GAT AAC CCT TTC CTC CAC TCC CAC AGG GAT		4429	
	Leu Pro Asp His Thr Ser Asp Asn Pro Phe Leu His Ser His Arg Asp			
	1360	1365	1370	
30	GAC CAA CGC TTG GTT ATT GGG AGA TGC CCC TCG GAC CCT TAC AAA CAC		4477	
	Asp Gln Arg Leu Val Ile Gly Arg Cys Pro Ser Asp Pro Tyr Lys His			
	1375	1380	1385	
35	TCG TTG CCA TCC CAG GCG GTG AAT GAC AGC TAT CTT CGG TCG TCC TTG		4525	
	Ser Leu Pro Ser Gln Ala Val Asn Asp Ser Tyr Leu Arg Ser Ser Leu			
	1390	1395	1400	1405
	AGG TCA ACG GCA TCG TAC TGT TCC AGG GAC AGT CGG GGC CAC AAT GAT		4573	
	Arg Ser Thr Ala Ser Tyr Cys Ser Arg Asp Ser Arg Gly His Asn Asp			
	1410	1415	1420	

	GTG TAT ATT TCG GAG CAT GTT ATG CCT TAT GCT GCA AAT AAG AAT AAT		4621
	Val Tyr Ile Ser Glu His Val Met Pro Tyr Ala Ala Asn Lys Asn Asn		
	1425	1430	1435
5			
	ATG TAC TCT ACC CCC AGG GTT TTA AAT TCC TGC AGC AAT AGA CGC GTG		4669
	Met Tyr Ser Thr Pro Arg Val Leu Asn Ser Cys Ser Asn Arg Arg Val		
	1440	1445	1450
10	TAC AAG GAA ATG CCT AGT ATC GAA TCT GAT GTT TAAAAATCTT CCATTAATGT		4722
	Tyr Lys Glu Met Pro Ser Ile Glu Ser Asp Val		
	1455	1460	146
15	TTTATCTATA GGGAAATACA CGTAATGGCC AATGTTCTGG AGGGTAAATG TTGGATGTCC		4782
	AATAGTGCCC TGCTAAGAGG AAGGAG		4808

(2) INFORMATION FOR SEQ ID NO:11:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1464 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

30	Met Gly Arg Val Gly Tyr Trp Thr Leu Leu Val Leu Pro Ala Leu Leu		
	1	5	10
			15
	Val Trp Arg Gly Pro Ala Pro Ser Ala Ala Ala Glu Lys Gly Pro Pro		
	20	25	30

35

Ala Leu Asn Ile Ala Val Met Leu Gly His Ser His Asp Val Thr Glu
 35 40 45

Arg Glu Leu Arg Thr Leu Trp Gly Pro Glu Gln Ala Ala Gly Leu Pro
5 50 55 60

Leu Asp Val Asn Val Val Ala Leu Leu Met Asn Arg Thr Asp Pro Lys
 65 70 75 80

10 Ser Leu Ile Thr His Val Cys Asp Leu Met Ser Gly Ala Arg Ile His
 85 90 95

Gly Leu Val Phe Gly Asp Asp Thr Asp Gln Glu Ala Val Ala Gln Met
 100 105 110

15
 Leu Asp Phe Ile Ser Ser His Thr Phe Val Pro Ile Leu Gly Ile His
 115 120 125

Gly Gly Ala Ser Met Ile Met Ala Asp Lys Asp Pro Thr Ser Thr Phe
20 130 135 140

Phe Gln Phe Gly Ala Ser Ile Gln Gln Ala Thr Val Met Leu Lys
 145 150 155 160

25 Ile Met Gln Asp Tyr Asp Trp His Val Phe Ser Leu Val Thr Thr Ile
 165 170 175

Phe Pro Gly Tyr Arg Glu Phe Ile Ser Phe Val Lys Thr Thr Val Asp
 180 185 190

30
 Asn Ser Phe Val Gly Trp Asp Met Gln Asn Val Ile Thr Leu Asp Thr
 195 200 205

Ser Phe Glu Asp Ala Lys Thr Gln Val Gln Leu Lys Lys Ile His Ser
35 210 215 220

Ser Val Ile Leu Leu Tyr Cys Ser Lys Asp Glu Ala Val Leu Ile Leu
225 230 235 240

5 Ser Glu Ala Arg Ser Leu Gly Leu Thr Gly Tyr Asp Phe Phe Trp Ile
245 250 255

Val Pro Ser Leu Val Ser Gly Asn Thr Glu Leu Ile Pro Lys Glu Phe
260 265 270

10 Pro Ser Gly Leu Ile Ser Val Ser Tyr Asp Asp Trp Asp Tyr Ser Leu
275 280 285

Glu Ala Arg Val Arg Asp Gly Ile Gly Ile Leu Thr Thr Ala Ala Ser
15 290 295 300

Ser Met Leu Glu Lys Phe Ser Tyr Ile Pro Glu Ala Lys Ala Ser Cys
305 310 315 320

20 Tyr Gly Gln Met Glu Arg Pro Glu Val Pro Met His Thr Leu His Pro
325 330 335

Phe Met Val Asn Val Thr Trp Asp Gly Lys Asp Leu Ser Phe Thr Glu
340 345 350

25 Glu Gly Tyr Gln Val His Pro Arg Leu Val Val Ile Val Leu Asn Lys
355 360 365

Asp Arg Glu Trp Glu Lys Val Gly Lys Trp Glu Asn His Thr Leu Ser
30 370 375 380

Leu Arg His Ala Val Trp Pro Arg Tyr Lys Ser Phe Ser Asp Cys Glu
385 390 395 400

35 Pro Asp Asp Asn His Leu Ser Ile Val Thr Leu Glu Glu Ala Pro Phe

	405	410	415	
	Val Ile Val Glu Asp Ile Asp Pro Leu Thr Glu Thr Cys Val Arg Asn			
	420	425	430	
5	Thr Val Pro Cys Arg Lys Phe Val Lys Ile Asn Asn Ser Thr Asn Glu			
	435	440	445	
	Gly Met Asn Val Lys Lys Cys Cys Lys Gly Phe Cys Ile Asp Ile Leu			
10	450	455	460	
	Lys Lys Leu Ser Arg Thr Val Lys Phe Thr Tyr Asp Leu Tyr Leu Val			
	465	470	475	480
15	Thr Asn Gly Lys His Gly Lys Val Asn Asn Val Trp Asn Gly Met			
	485	490	495	
	Ile Gly Glu Val Val Tyr Gln Arg Ala Val Met Ala Val Gly Ser Leu			
	500	505	510	
20	Thr Ile Asn Glu Glu Arg Ser Glu Val Val Asp Phe Ser Val Pro Phe			
	515	520	525	
	Val Glu Thr Gly Ile Ser Val Met Val Ser Arg Ser Asn Gly Thr Val			
25	530	535	540	
	Ser Pro Ser Ala Phe Leu Glu Pro Phe Ser Ala Ser Val Trp Val Met			
	545	550	555	560
30	Met Phe Val Met Leu Leu Ile Val Ser Ala Ile Ala Val Trp Val Leu			
	565	570	575	
	Asp Tyr Ser Ser Pro Val Gly Tyr Asn Arg Asn Leu Ala Lys Gly Lys			
	580	585	590	
35				

Ala Pro His Gly Pro Ser Phe Thr Ile Gly Lys Ala Ile Trp Leu Leu
 595 600 605

Trp Gly Leu Val Phe Asn Asn Ser Val Pro Val Gln Asn Pro Lys Gly
5 610 615 620

Thr Thr Ser Lys Ile Met Val Ser Val Trp Ala Phe Phe Ala Val Ile
 625 630 635 640

10 Phe Leu Ala Ser Tyr Thr Ala Asn Leu Ala Ala Phe Met Ile Gln Glu
 645 650 655

Glu Phe Val Asp Gln Val Thr Gly Leu Ser Asp Lys Lys Phe Gln Arg
 660 665 670

15
 Pro His Asp Tyr Ser Pro Pro Phe Arg Phe Gly Thr Val Pro Asn Gly
 675 680 685

Ser Thr Glu Arg Asn Ile Arg Asn Asn Tyr Pro Tyr Met His Gln Tyr
20 690 695 700

Met Thr Lys Phe Asn Gln Lys Gly Val Glu Asp Ala Leu Val Ser Leu
 705 710 715 720

25 Lys Thr Gly Lys Leu Asp Ala Phe Ile Tyr Asp Ala Ala Val Leu Asn
 725 730 735

Tyr Lys Ala Gly Arg Asp Glu Gly Cys Lys Leu Val Thr Ile Gly Ser
 740 745 750

30
 Gly Tyr Ile Phe Ala Thr Thr Gly Tyr Gly Ile Ala Leu Gln Lys Gly
 755 760 765

Ser Pro Trp Lys Arg Gln Ile Asp Leu Ala Leu Leu Gln Phe Val Gly
35 770 775 780

Asp Gly Glu Met Glu Glu Leu Glu Thr Leu Trp Leu Thr Gly Ile Cys
785 790 795 800

5 His Asn Glu Lys Asn Glu Val Met Ser Ser Gln Leu Asp Ile Asp Asn
805 810 815

Met Ala Gly Val Phe Tyr Met Leu Ala Ala Ala Met Ala Leu Ser Leu
820 825 830

10 Ile Thr Phe Ile Trp Glu His Leu Phe Tyr Trp Lys Leu Arg Phe Cys
835 840 845

Phe Thr Gly Val Cys Ser Asp Arg Pro Gly Leu Leu Phe Ser Ile Ser
15 850 855 860

Arg Gly Ile Tyr Ser Cys Ile His Gly Val His Ile Glu Glu Lys Lys
865 870 875 880

20 Lys Ser Pro Asp Phe Asn Leu Thr Gly Ser Gln Ser Asn Met Leu Lys
885 890 895

Leu Leu Arg Ser Ala Lys Asn Ile Ser Ser Met Ser Asn Met Asn Ser
900 905 910

25 Ser Arg Met Asp Ser Pro Lys Arg Ala Ala Asp Phe Ile Gln Arg Gly
915 920 925

Ser Leu Ile Met Asp Met Val Ser Asp Lys Gly Asn Leu Met Tyr Ser
30 930 935 940

Asp Asn Arg Ser Phe Gln Gly Lys Glu Ser Ile Phe Gly Asp Asn Met
945 950 955 960

35 Asn Glu Leu Gln Thr Phe Val Ala Asn Arg Gln Lys Asp Asn Leu Asn

965

970

975

Asn Tyr Val Phe Gln Gly Gln His Pro Leu Thr Leu Asn Glu Ser Asn

980

985

990

5

Pro Asn Thr Val Glu Val Ala Val Ser Thr Glu Ser Lys Ala Asn Ser

995

1000

1005

Arg Pro Arg Gln Leu Trp Lys Lys Ser Val Asp Ser Ile Arg Gln Asp

10

1010

1015

1020

Ser Leu Ser Gln Asn Pro Val Ser Gln Arg Asp Glu Ala Thr Ala Glu

1025

1030

1035

1040

15

Asn Arg Thr His Ser Leu Lys Ser Pro Arg Tyr Leu Pro Glu Glu Met

1045

1050

1055

Ala His Ser Asp Ile Ser Glu Thr Ser Asn Arg Ala Thr Cys His Arg

1060

1065

1070

20

Glu Pro Asp Asn Ser Lys Asn His Lys Thr Lys Asp Asn Phe Lys Arg

1075

1080

1085

Ser Val Ala Ser Lys Tyr Pro Lys Asp Cys Ser Glu Val Glu Arg Thr

25

1090

1095

1100

Tyr Leu Lys Thr Lys Ser Ser Pro Arg Asp Lys Ile Tyr Thr Ile

1105

1110

1115

1120

30

Asp Gly Glu Lys Glu Pro Gly Phe His Leu Asp Pro Pro Gln Phe Val

1125

1130

1135

Glu Asn Val Thr Leu Pro Glu Asn Val Asp Phe Pro Asp Pro Tyr Gln

1140

1145

1150

35

Asp Pro Ser Glu Asn Phe Arg Lys Gly Asp Ser Thr Leu Pro Met Asn
1155 1160 1165

Arg Asn Pro Leu His Asn Glu Glu Gly Leu Ser Asn Asn Asp Gln Tyr
5 1170 1175 1180

Lys Leu Tyr Ser Lys His Phe Thr Leu Lys Asp Lys Gly Ser Pro His
1185 1190 1195 1200

10 Ser Glu Thr Ser Glu Arg Tyr Arg Gln Asn Ser Thr His Cys Arg Ser
1205 1210 1215

Cys Leu Ser Asn Met Pro Thr Tyr Ser Gly His Phe Thr Met Arg Ser
1220 1225 1230

15 Pro Phe Lys Cys Asp Ala Cys Leu Arg Met Gly Asn Leu Tyr Asp Ile
1235 1240 1245

Asp Glu Asp Gln Met Leu Gln Glu Thr Gly Asn Pro Ala Thr Gly Glu
20 1250 1255 1260

Gln Val Tyr Gln Gln Asp Trp Ala Gln Asn Asn Ala Leu Gln Leu Gln
1265 1270 1275 1280

25 Lys Asn Lys Leu Arg Ile Ser Arg Gln His Ser Tyr Asp Asn Ile Val
1285 1290 1295

Asp Lys Pro Arg Glu Leu Asp Leu Ser Arg Pro Ser Arg Ser Ile Ser
1300 1305 1310

30 Leu Lys Asp Arg Glu Arg Leu Leu Glu Gly Asn Phe Tyr Gly Ser Leu
1315 1320 1325

Phe Ser Val Pro Ser Ser Lys Leu Ser Gly Lys Lys Ser Ser Leu Phe
35 1330 1335 1340

Pro Gln Gly Leu Glu Asp Ser Lys Arg Ser Lys Ser Leu Leu Pro Asp
1345 1350 1355 1360

5 His Thr Ser Asp Asn Pro Phe Leu His Ser His Arg Asp Asp Gln Arg
1365 1370 1375

Leu Val Ile Gly Arg Cys Pro Ser Asp Pro Tyr Lys His Ser Leu Pro
1380 1385 1390

10 Ser Gln Ala Val Asn Asp Ser Tyr Leu Arg Ser Ser Leu Arg Ser Thr
1395 1400 1405

Ala Ser Tyr Cys Ser Arg Asp Ser Arg Gly His Asn Asp Val Tyr Ile
1410 1415 1420

Ser Glu His Val Met Pro Tyr Ala Ala Asn Lys Asn Asn Met Tyr Ser
1425 1430 1435 1440

20 Thr Pro Arg Val Leu Asn Ser Cys Ser Asn Arg Arg Val Tyr Lys Glu
1445 1450 1455

Met Pro Ser Ile Glu Ser Asp Val
1460

25

(2) INFORMATION FOR SEQ ID NO:12:

30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 74 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

35 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CGAGGGAGGC GGCGGGCGCG GACTCTCTTC GCGGGCGCAG CGCCCGTTCC CCCTCGGACC	60
5 CTCCGGTGGAA CATG	74

(2) INFORMATION FOR SEQ ID NO:13:

10

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5538 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both

15

- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

20

- (A) NAME/KEY: CDS
- (B) LOCATION: 210..4664

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

25

TTGAATTGCA ATCTCTTCAA GACACAAGAT TAAAACAAAA TTTAAGCTAA ATTGGATTTC

60

AAATTATCTT CGGTTCATTT ATCCTTCGTC TTTCTTATGT GGATATGCAA GCGAGAAGAA

120

GGGACTGGAC ATTCCCCAACAA TGDTCACTCC CTTAATCTGT CCGTGTAGAG GTTTGGGTTC

180

30

TACAAACCAA GGGAGTCGAC GAGTTGAAG ATG AAG CCC AGA GCG GAG TGC TGT

233

Met Lys Pro Arg Ala Glu Cys Cys

1

5

35

TCT CCC AAG TTC TGG TTG CTG TTG GCC GTC CTG GCC GTG TCA GGC AGC

261

Ser Pro Lys Phe Trp Leu Val Leu Ala Val Leu Ala Val Ser Gly Ser
 10 15 20

AGA GCT CGT TCT CAG AAG AGC CCC CCC AGC ATT GGC ATT GCT GTC ATC 329
 5 Arg Ala Arg Ser Gln Lys Ser Pro Pro Ser Ile Gly Ile Ala Val Ile
 25 30 35 40

CTC GTG GGC ACT TCC GAC GAG GTG GGC ATC AAG GAT GCC CAC GAG AAA 377
 Leu Val Gly Thr Ser Asp Glu Val Ala Ile Lys Asp Ala His Glu Lys
 10 45 50 55

GAT GAT TTC CAC CAT CTC TCC GTG GTA CCC CGG GTG GAA CTG GTA GCC 425
 Asp Asp Phe His His Leu Ser Val Val Pro Arg Val Glu Leu Val Ala
 60 65 70

15 ATG AAT GAG ACC GAC CCA AAG AGC ATC ATC ACC CGC ATC TGT GAT CTC 473
 Met Asn Glu Thr Asp Pro Lys Ser Ile Ile Thr Arg Ile Cys Asp Leu
 75 80 85

20 ATG TCT GAC CGG AAG ATC CAG GGG GTG GTG TTT GCT GAT GAC ACA GAC 521
 Met Ser Asp Arg Lys Ile Gln Gly Val Val Phe Ala Asp Asp Thr Asp
 90 95 100

CAG GAA GCC ATC GCC CAG ATC CTC GAT TTC ATT TCA GCA CAG ACT CTC 569
 25 Gln Glu Ala Ile Ala Gln Ile Leu Asp Phe Ile Ser Ala Gln Thr Leu
 105 110 115 120

ACC CCG ATC CTG GGC ATC CAC GGG GGC TCC TCT ATG ATA ATG GCA GAT 617
 Thr Pro Ile Leu Gly Ile His Gly Ser Ser Met Ile Met Ala Asp
 30 125 130 135

AAG GAT GAA TCC TCC ATG TTC TTC CAG TTT GGC CCA TCA ATT GAA CAG 665
 Lys Asp Glu Ser Ser Met Phe Phe Gln Phe Gly Pro Ser Ile Glu Gln
 140 145 150

	CAA GCT TCC GTA ATG CTC AAC ATC ATG GAA GAA TAT GAC TGG TAC ATC		713	
	Gln Ala Ser Val Met Leu Asn Ile Met Glu Glu Tyr Asp Trp Tyr Ile			
	155	160	165	
5	TTT TCT ATC GTC ACC ACC TAT TTC CCT GGC TAC GAG GAC TTT GTA AAC		761	
	Phe Ser Ile Val Thr Thr Tyr Phe Pro Gly Tyr Gln Asp Phe Val Asn			
	170	175	180	
10	AAG ATC CGC AGC ACC ATT GAG AAT AGC TTT GTG GGC TGG GAG CTA GAG		809	
	Lys Ile Arg Ser Thr Ile Glu Asn Ser Phe Val Gly Trp Glu Leu Glu			
	185	190	195	200
15	GAG GTC CTC CTA CTG GAC ATG TCC CTG GAC GAT GGA GAT TCT AAG ATC		857	
	Glu Val Leu Leu Leu Asp Met Ser Leu Asp Asp Gly Asp Ser Lys Ile			
	205	210	215	
20	CAG AAT CAG CTC AAG AAA CTT CAA AGC CCC ATC ATT CTT CTT TAC TGT		905	
	Gln Asn Gln Leu Lys Lys Leu Gln Ser Pro Ile Ile Leu Leu Tyr Cys			
	220	225	230	
25	ACC AAG GAA GAA GCC ACC TAC ATC TTT GAA GTG GGC AAC TCA GTA GGG		953	
	Thr Lys Glu Ala Thr Tyr Ile Phe Glu Val Ala Asn Ser Val Gly			
	235	240	245	
30	CTG ACT GGC TAT GGC TAC ACG TGG ATC GTG CCC AGT CTG GTG GCA GGG		1001	
	Leu Thr Gly Tyr Gly Tyr Thr Trp Ile Val Pro Ser Leu Val Ala Gly			
	250	255	260	
35	GAT ACA GAC ACA GTG CCT GCG GAG TTC CCC ACT GGG CTC ATC TCT GTA		1049	
	Asp Thr Asp Thr Val Pro Ala Glu Phe Pro Thr Gly Leu Ile Ser Val			
	265	270	275	280
	TCA TAT GAT GAA TGG GAC TAT GGC CTC CCC CCC AGA GTG AGA GAT GGA		1097	
	Ser Tyr Asp Glu Trp Asp Tyr Gly Leu Pro Pro Arg Val Arg Asp Gly			
	285	290	295	

	ATT GCC ATA ATC ACC ACT GCT GCT TCT GAC ATG CTG TCT GAG CAC AGC		1145	
	Ile Ala Ile Ile Thr Thr Ala Ala Ser Asp Met Leu Ser Glu His Ser			
	300	305	310	
5				
	TTC ATC CCT GAG CCC AAA AGC AGT TGT TAC AAC AAC CAC GAG AAG AGA		1193	
	Phe Ile Pro Glu Pro Lys Ser Ser Cys Tyr Asn Thr His Glu Lys Arg			
	315	320	325	
10	ATC TAC CAG TCC AAT ATG CTA AAT AGG TAT CTG ATC AAT GTC ACT TTT		1241	
	Ile Tyr Gln Ser Asn Met Leu Asn Arg Tyr Leu Ile Asn Val Thr Phe			
	330	335	340	
	GAG GGG AGG AAT TTG TCC TTC AGT GAA GAT GGC TAC CAG ATG CAC CCG		1289	
15	Glu Gly Arg Asn Leu Ser Phe Ser Glu Asp Gly Tyr Gln Met His Pro			
	345	350	355	360
	AAA CTG GTG ATA ATT CTT CTG AAC AAG GAG AGG AAG TGG GAA AGG GTG		1337	
	Lys Leu Val Ile Ile Leu Leu Asn Lys Glu Arg Lys Trp Glu Arg Val			
20	365	370	375	
	GGG AAG TGG AAA GAC AAG TCC CTG CAG ATG AAG TAC TAT GTG TGG CCC		1385	
	Gly Lys Trp Lys Asp Lys Ser Leu Gln Met Lys Tyr Tyr Val Trp Pro			
	380	385	390	
25				
	CGA ATG TGT CCA GAG ACT GAA GAG CAG GAG GAT GAC CAT CTG AGC ATT		1433	
	Arg Met Cys Pro Glu Thr Glu Glu Gln Glu Asp Asp His Leu Ser Ile			
	395	400	405	
30	GTG ACC CTG GAG GAG GCA CCA TTT GTC ATT GTG GAA AGT GTG GAC CCT		1481	
	Val Thr Leu Glu Glu Ala Pro Phe Val Ile Val Glu Ser Val Asp Pro			
	410	415	420	
	CTG AGT GGA ACC TGC ATG AGG AAC ASA GTC CCC TGC CAA AAA CGE ATA		1529	
35	Leu Ser Gly Thr Cys Met Arg Asn Thr Val Pro Cys Gln Lys Arg Ile			

	425	430	435	440	
	GTC ACT GAG AAT AAA ACA GAC GAG GAG CCG GGT TAC ATC AAA AAA TGC				1577
	Val Thr Glu Asn Lys Thr Asp Glu Glu Pro Gly Tyr Ile Lys Lys Cys				
5	445	450	455		
	TGC AAG GGG TTC TGT ATT GAC ATC CTT AAG AAA ATT TCT AAA TCT GTG				1625
	Cys Lys Gly Phe Cys Ile Asp Ile Leu Lys Lys Ile Ser Lys Ser Val				
	460	465	470		
10					
	AAG TTC ACC TAT GAC CTT TAC CTG GTT ACC AAT GGC AAG CAT GGG AAG				1673
	Lys Phe Thr Tyr Asp Leu Tyr Leu Val Thr Asn Gly Lys His Gly Lys				
	475	480	485		
15	AAA ATC AAT GGA ACC TGG AAT GGT ATG ATT GGA GAG GTG GTC ATG AAG				1721
	Lys Ile Asn Gly Thr Trp Asn Gly Met Ile Gly Glu Val Val Met Lys				
	490	495	500		
	AGG GGC TAC ATG GCA GTG GGC TCA CTC ACC ATC AAT GAG GAA CGA TCG				1769
20	Arg Ala Tyr Met Ala Val Gly Ser Leu Thr Ile Asn Glu Glu Arg Ser				
	505	510	515	520	
	GAG GTG GTC GAC TTC TCT GTG CCC TTC ATA GAG ACA GGC ATC AGT GTC				1817
	Glu Val Val Asp Phe Ser Val Pro Phe Ile Glu Thr Gly Ile Ser Val				
25	525	530	535		
	ATG GTG TCA CGC AGC AAT GGG ACT GTC TCA CCT TCT GCC TTC TTA GAG				1865
	Met Val Ser Arg Ser Asn Gly Thr Val Ser Pro Ser Ala Phe Leu Glu				
	540	545	550		
30					
	CCA TTC AGC GCT GAC GTC TGG GTG ATG ATG TTT GTG ATG CTG CTC ATC				1913
	Pro Phe Ser Ala Asp Val Trp Val Met Met Phe Val Met Leu Leu Ile				
	555	560	565		
35	GTC TCA GCC GTG GCT GTC TTT GTC TTT GAG TAC TTC AGC CCT GTG GGT				1961

	Val Ser Ala Val Ala Val Phe Val Phe Glu Tyr Phe Ser Pro Val Gly			
	570	575	580	
	TAT AAC AGG TGC CTC GCT GAT GGC AGA GAG CCT GGT GGA CCC TCT TTC 2009			
5	Tyr Asn Arg Cys Leu Ala Asp Gly Arg Glu Pro Gly Gly Pro Ser Phe			
	585	590	595	600
	ACC ATC GGC AAA GCT ATT TGG TTG CTC TGG GGT CTG GTG TTT AAC AAC 2057			
	Thr Ile Gly Lys Ala Ile Trp Leu Leu Trp Gly Leu Val Phe Asn Asn			
10	605	610	615	
	TCC GTA CCT GTG CAG AAC CCA AAG GGG ACC ACC TCC AAG ATC ATG GTG 2105			
	Ser Val Pro Val Gln Asn Pro Lys Gly Thr Thr Ser Lys Ile Met Val			
	620	625	630	
15				
	TCA GTG TGG GCC TTC TTT GCT GTC ATC TTC CTG GCC AGC TAC ACT GCC 2153			
	Ser Val Trp Ala Phe Phe Ala Val Ile Phe Leu Ala Ser Tyr Thr Ala			
	635	640	645	
20	AAC TTA GCT GCC TTC ATG ATC CAA GAG GAA TAT GTG GAC CAG GTT TCT 2201			
	Asn Leu Ala Ala Phe Met Ile Gln Glu Glu Tyr Val Asp Gln Val Ser			
	650	655	660	
	GGC CTG AGC GAC AAA AAG TTC CAG AGA CCT AAT GAC TTC TCA CCC CCT 2249			
25	Gly Leu Ser Asp Lys Lys Phe Gln Arg Pro Asn Asp Phe Ser Pro Pro			
	665	670	675	680
	TTC CGC TTT GGG ACC GTG CCC AAC GGC AGC ACA GAG AGA AAT ATT CGC 2297			
	Phe Arg Phe Gly Thr Val Pro Asn Gly Ser Thr Gli Arg Asn Ile Arg			
30	685	690	695	
	AAT AAC TAT GCA GAA ATG CAT GCC TAC ATG GGA AAG TTC AAC CAG AGG 2345			
	Asn Asn Tyr Ala Glu Met His Ala Tyr Met Gly Lys Phe Asn Gln Arg			
	700	705	710	

	GGT GTC GAT GAT GCA TTG CTC TCC CTG AAA ACA GGG AAA CTG GAT GCC		1393
	Gly Val Asp Asp Ala Leu Leu Ser Leu Lys Thr Gly Lys Leu Asp Ala		
	715	720	725
5	TTC ATC TAT GAT GCA GCA GTG CTG AAC TAT ATG GCA GGC AGA GAT GAA		2441
	Phe Ile Tyr Asp Ala Ala Val Leu Asn Tyr Met Ala Gly Arg Asp Glu		
	730	735	740
	GGC TGC AAG CTG GTG ACC ATT GGC AGT GGG AAG GTC TTT GCT TCC ACT		2489
10	Gly Cys Lys Leu Val Thr Ile Gly Ser Gly Lys Val Phe Ala Ser Thr		
	745	750	755
	760		
	GGC TAT GGC ATT GCC ATC CAA AAA GAT TCT GGG TGG AAG CGC CAG GTG		2537
	Gly Tyr Gly Ile Ala Ile Gln Lys Asp Ser Gly Trp Lys Arg Gln Val		
15	765	770	775
	GAC CTT GCT ATC CTG CAG CTC TTT GGA GAT GGG GAG ATG GAA GAA CTG		2585
	Asp Leu Ala Ile Leu Gln Leu Phe Gly Asp Gly Glu Met Glu Leu		
	780	785	790
20			
	GAA GCT CTC TGG CTC ACT GGC ATT TGT CAC AAT GAG AAG AAT GAG GTC		2633
	Gly Ala Leu Trp Leu Thr Gly Ile Cys His Asn Glu Lys Asn Glu Val		
	795	800	805
25	ATG AGC AGC CAG CTG GAC ATT GAC AAC ATG GCA GGG GTC TTC TAC ATG		2681
	Met Ser Ser Gln Leu Asp Ile Asp Asn Met Ala Gly Val Phe Tyr Met		
	810	815	820
	TTG GGG GCG GCC ATG GCT CTC AGC CTC ATC ACC TTC ATC TGC GAA CAC		2729
30	Leu Gly Ala Ala Met Ala Leu Ser Leu Ile Thr Phe Ile Cys Glu His		
	825	830	835
	840		
	CTT TTC TAT TGG CAG TTC CGA CAT TGC TTT ATG GGT GTC TGT TCT GGC		2777
	Leu Phe Tyr Trp Gln Phe Arg His Cys Phe Met Gly Val Cys Ser Gly		
35	845	850	855

	AAG CCT GGC ATG GTC TTC TCC ATC AGC AGA GGT ATC TAC AGC TGC ATC		2825
	Lys Pro Gly Met Val Phe Ser Ile Ser Arg Gly Ile Tyr Ser Cys Ile		
	860	865	870
5			
	CAT GGG GTG GCG ATC GAG GAG CGC CAG TCT GTA ATG AAC TCC CCC ACC		2873
	His Gly Val Ala Ile Glu Glu Arg Gln Ser Val Met Asn Ser Pro Thr		
	875	880	885
10	GCA ACG ATG AAC AAC ACA CAC TCC AAC ATC CTG CGC CTG CGC ACG		2921
	Ala Thr Met Asn Asn Thr His Ser Asn Ile Leu Arg Leu Leu Arg Thr		
	890	895	900
	GCC AAG AAC ATG GCT AAC CTG TCT GGT GTG AAT GGC TCA CCG CAG AGC		2969
15	Ala Lys Asn Met Ala Asn Leu Ser Gly Val Asn Gly Ser Pro Gln Ser		
	905	910	915
			920
	GCC CTG GAC TTC ATC CGA CGG GAG TCA TCC GTC TAT GAC ATC TCA GAG		3017
	Ala Leu Asp Phe Ile Arg Arg Glu Ser Ser Val Tyr Asp Ile Ser Glu		
20	925	930	935
	CAC CGC CGC AGC TTC ACG CAT TCT GAC TGC AAA TCC TAC AAC AAC CCG		3065
	His Arg Arg Ser Phe Thr His Ser Asp Cys Lys Ser Tyr Asn Asn Pro		
	940	945	950
25			
	CCC TGT GAG GAG AAC CTC TTC AGT GAC TAC ATC AGT GAG GTA GAG AGA		3113
	Pro Cys Glu Glu Asn Leu Phe Ser Asp Tyr Ile Ser Glu Val Glu Arg		
	955	960	965
30	ACG TTC GGG AAC CTG CAG CTG AAG GAC AGC AAC GTG TAC CAA GAT CAC		3161
	Thr Phe Gly Asn Leu Gln Leu Lys Asp Ser Asn Val Tyr Gln Asp His		
	970	975	980
35	TAC CAC CAT CAC CAC CGG CCC CAT AGT ATT GGC AGT GCC AGC TCC ATC		3209
	Tyr His His His Arg Pro His Ser Ile Gly Ser Ala Ser Ser Ile		

	985	990	995	1000	
	GAT GGG CTC TAC GAC TGT GAC AAC CCA CCC TTC ACC ACC CAG TCC AGG				3257
	Asp Gly Leu Tyr Asp Cys Asp Asn Pro Pro Phe Thr Thr Gln Ser Arg				
5	1015	1010	1015		
	TCC ATC AGC AAG AAG CCC CTG GAC ATC GGC CTC CCC TCC AAG CAC				3305
	Ser Ile Ser Lys Lys Pro Leu Asp Ile Gly Leu Pro Ser Ser Lys His				
	1020	1025	1030		
10					
	AGC CAG CTC AGT GAC CTG TAC GGC AAA TTC TCC TTC AAG AGC GAC CGC				3353
	Ser Gln Leu Ser Asp Leu Tyr Gly Lys Phe Ser Phe Lys Ser Asp Arg				
	1035	1040	1045		
15	TAC AGT GGC CAC GAC GAC TTG ATC CGC TCC GAT GTC TCT GAC ATC TCA				3401
	Tyr Ser Gly His Asp Asp Leu Ile Arg Ser Asp Val Ser Asp Ile Ser				
	1050	1055	1060		
	ACC CAC ACC GTC ACC TAT GGG AAC ATC GAG GGC AAT GCC GCC AAG AAG				3449
20	Thr His Thr Val Thr Tyr Gly Asn Ile Glu Gly Asn Ala Ala Lys Arg				
	1065	1070	1075	1080	
	CGT AAG CAG CAA TAT AAG GAC AGC CTG AAG AAG CGG CCT GCC TCG GCC				3497
	Arg Lys Gln Gln Tyr Lys Asp Ser Leu Lys Lys Arg Pro Ala Ser Ala				
25	1085	1090	1095		
	AAG TCC CGC AGG GAG TTT GAC GAG ATC GAG CTG GCC TAC CGT CGC CGA				3545
	Lys Ser Arg Arg Glu Phe Asp Glu Ile Glu Leu Ala Tyr Arg Arg Arg				
	1100	1105	1110		
30					
	CCG CCC CGC TCC CCT GAC CAC AAG CGC TAC TTC AGG GAC AAG GAA GGG				3593
	Pro Pro Arg Ser Pro Asp His Lys Arg Tyr Phe Arg Asp Lys Glu Gly				
	1115	1120	1125		
35	CTA CGG GAC TTC TAC CTG GAC CAG TTC CGA ACA AAG GAG AAC TCA CCC				3641

Leu Arg Asp Phe Tyr Leu Asp Gln Phe Arg Thr Lys Glu Asn Ser Pro
 1130 1135 1140

CAC TGG GAG CAC GTA GAC CTG ACC GAC ATC TAC AAG GAG CGG AGT GAT 3689
 5 His Trp Glu His Val Asp Leu Thr Asp Ile Tyr Lys Glu Arg Ser Asp
 1145 1150 1155 1160

GAC TTT AAG CGC GAC TCC ATC AGC GGA GGA GGG CCC TGT ACC AAC AGG 3737
 Asp Phe Lys Arg Asp Ser Ile Ser Gly Gly Pro Cys Thr Asn Arg
 10 1165 1170 1175

TCT CAC ATC AAG CAC GGG ACG GGC GAC AAA CAC GGC GTG GTC AGC GGG 3785
 Ser His Ile Lys His Gly Thr Gly Asp Lys His Gly Val Val Ser Gly
 1180 1185 1190

15 GTA CCT GCA CCT TGG GAG AAG AAC CTG ACC AAC GTG GAG TGG GAG GAC 3833
 Val Pro Ala Pro Trp Glu Lys Asn Leu Thr Asn Val Glu Trp Glu Asp
 1195 1200 1205

20 CGG TCC GGG GGC AAC TTC TGC CGC ACG TGT CCC TCC AAG CTG CAC AAC 3881
 Arg Ser Gly Asn Phe Cys Arg Ser Cys Pro Ser Lys Leu His Asn
 1210 1215 1220

TAC TCC ACG ACG GTG ACG GGT CAG AAC TCG GGC AGG CAG GCG TGC ATC 3929
 25 Tyr Ser Thr Thr Val Thr Gly Gln Asn Ser Gly Arg Gln Ala Cys Ile
 1225 1230 1235 1240

CGG TGT GAG GCT TGC AAG AAA GCA GGC AAC CTG TAT GAC ATC AGT GAG 3977
 Arg Cys Glu Ala Cys Lys Lys Ala Gly Asn Leu Tyr Asp Ile Ser Glu
 30 1245 1250 1255

GAC AAC TCC CTG CAG GAA CTG GAC CAG CGG GCT GGC CCA GTG GCG GTG 4025
 Asp Asn Ser Leu Gln Glu Leu Asp Gln Pro Ala Ala Pro Val Ala Val
 1260 1265 1270

35

	ACG TCA AAC GCC TCC ACC ACT AAG TAC CCT CAG AGC CCG ACT AAT TCC	4173
	Thr Ser Asn Ala Ser Thr Thr Lys Tyr Pro Gln Ser Pro Thr Asn Ser	
	1275 1280 1285	
5	AAG GCC CAG AAG AAC CGG AAC AAA CTG CGC CGG CAG CAC TCC TAC	4121
	Lys Ala Gln Lys Lys Asn Arg Asn Lys Leu Arg Arg Gln His Ser Tyr	
	1290 1295 1300	
	GAC ACC TTC GTG GAC CTG CAG AAG GAA GAA GCC GCC CTG GCC CGG CGC	4169
10	Asp Thr Phe Val Asp Leu Gln Lys Glu Glu Ala Ala Leu Ala Pro Arg	
	1305 1310 1315 1320	
	AGC GTA AGC CTG AAA GAC AAG GGC CGA TTC ATG GAT GGG AGC CCC TAC	4217
	Ser Val Ser Leu Lys Asp Lys Gly Arg Phe Met Asp Gly Ser Pro Tyr	
15	1325 1330 1335	
	GCC CAC ATG TTT GAG ATG TCA GCT GCC GAG AGC ACC TTP GCC AAC AAC	4265
	Ala His Met Phe Glu Met Ser Ala Gly Glu Ser Thr Phe Ala Asn Asn	
	1340 1345 1350	
20	AAG TCC TCA GTG CCC ACT GCC GGA CAT CAC CAC CAC AAC AAC CCC GGC	4313
	Lys Ser Ser Val Pro Thr Ala Gly His His His His Asn Asn Pro Gly	
	1355 1360 1365	
25	GGC GGG TAC ATG CTC AGC AAG TCG CTC TAC CCT GAC CGG GTC ACG CAA	4361
	Gly Gly Tyr Met Leu Ser Lys Ser Leu Tyr Pro Asp Arg Val Thr Gln	
	1370 1375 1380	
	AAC CCT TTC ATC CCC ACT TTT GGG GAC GAC CAG TGC TTG CTC CAT GGC	4409
30	Asn Pro Phe Ile Pro Thr Phe Gly Asp Asp Gln Cys Leu Leu His Gly	
	1385 1390 1395 1400	
	AGC AAA TCC TAC TTC TTC AGG CAG CGC ACG GTG GCG GCG TCG AAA	4457
	Ser Lys Ser Tyr Phe Phe Arg Gln Pro Thr Val Ala Gly Ala Ser Lys	
35	1415 1410 1415	

	GCC AGG CCG GAC TTC CGG GCC CTT GTC ACC AAC AAG CCG GTG GTC TCG	4515
	Ala Arg Pro Asp Phe Arg Ala Ile Val Thr Asn Lys Pro Val Val Ser	
	1420 1425 1430	
5		
	GCC CTT CAT GGG GCC GTG CCA GCC CGT TTC CAG AAG GAC ATC TGT ATA	4553
	Ala Leu His Gly Ala Val Pro Ala Arg Phe Gln Lys Asp Ile Cys Ile	
	1435 1440 1445	
10	GGG AAC CAG TCC AAC CCC TGT GTG CCT AAC AAC ACA AAC CCC AGG GCT	4601
	Gly Asn Gln Ser Asn Pro Cys Val Pro Asn Asn Thr Asn Pro Arg Ala	
	1450 1455 1460	
	TTC AAT GGC TCC AGC AAT GGG CAT GTT TAT GAG AAA CTT TCT AGT ATT	4649
15	Phe Asn Gly Ser Ser Asn Gly His Val Tyr Glu Lys Leu Ser Ser Ile	
	1465 1470 1475 1480	
	GAG TCT GAT GTC TGAGTGAGGG AACAGAGAGG TTAAGGTGGG TACGGGAGGG	4701
	Glu Ser Asp Val	
20	148	
	TAAGGCTGTG GGTCGCGTGA TGCGCATGTC ACGGAGGGTG ACGGGGGTGA ACTTGGTTCC	4761
	CATTTGCTCC TTTCTTGTAA TAATTATTT ATGGGATCCT GGAGTTCTGG TTCTACTGG	4821
25	GGGCAACCT GGTGACCAGC ACCATCTCTC CTCTTTCTCA CAGTTCTCTC CTCTTTCCCC	4881
	CCGCTGTCAG CCATTCCTGT TCCCCTGAGA TGATGCCATG GGGCTCTCA GCAGGGGAGG	4941
30	GTAGAGCGGA GAAAGGAAAG GCTGCATGEG GGCTTCCCTCC TGGTGTGCAA GAGCTCTTG	5001
	ATATCTTGTG TGAGTGAAGC TGGGAGAACC AAAAAGAGGC TAGTGAGCA CAAAGGTAGC	5061
	TTTTCCAAA TTGATCTTTT CATTTAGGTG AGGAAGCAAAGGAGCTATG TGAGACCATT	5121
35		

TAGCACACTG CTTGTGAAAG GAAAGAGGCT CTGGCTAAAT TCATGCTGCT TAGATGACAT 5181
 CTGCTCTAGGA ATCATGTCGC AANGCAGAGGT TGGGAGGCCA TTTGTGTTA TATATAAGCC 5241
 5 CAAAAATGCT TGCTTCAACC CCATGAGACT CGATAGTGTT GGTGAACAGA ACCCAAGGTC 5301
 ATTGGTGGCA GAGTGGATTG TTGAACAAAC TGGAAAGTAC GTTATGATAG TGTCCCCCGG 5361
 TGCCTTGGGG ACAAGAGCAG GTGGATTGTG CGTGCATGTG TGTTCATGCA CACTTGACCC 5421
 10 CATGTGTAGT CAGGTGCCTC AAGAGAAGGC AACCTTGACT CTTTCGTTGA ATTTGCATCT 5481
 CTTCAAGACA CAAGATTAAA ACAAAATTAA CGCTAAATTG GATTTTAAAT TATCTTC 5538
 15
 (2) INFORMATION FOR SEQ ID NO:14:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1484 amino acids
 20 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: protein
 25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
 Met Lys Pro Arg Ala Glu Cys Cys Ser Pro Lys Phe Trp Leu Val Leu
 1 5 10 15
 30 Ala Val Leu Ala Val Ser Gly Ser Arg Ala Arg Ser Gin Lys Ser Pro
 20 25 30
 Pro Ser Ile Gly Ile Ala Val Ile Leu Val Gly Thr Ser Asp Glu Val
 35 40 45
 35

Ala Ile Lys Asp Ala His Glu Lys Asp Asp Phe His His Leu Ser Val
50 55 60

Val Pro Arg Val Glu Leu Val Ala Met Asn Glu Thr Asp Pro Lys Ser
5 65 70 75 80

Ile Ile Thr Arg Ile Cys Asp Leu Met Ser Asp Arg Lys Ile Gln Gly
85 90 95

10 Val Val Phe Ala Asp Asp Thr Asp Gln Glu Ala Ile Ala Gln Ile Leu
100 105 110

Asp Phe Ile Ser Ala Gln Thr Leu Thr Pro Ile Leu Gly Ile His Gly
115 120 125

15 Gly Ser Ser Met Ile Met Ala Asp Lys Asp Glu Ser Ser Met Phe Phe
130 135 140

Gln Phe Gly Pro Ser Ile Glu Gln Gln Ala Ser Val Met Leu Asn Ile
20 145 150 155 160

Met Glu Glu Tyr Asp Trp Tyr Ile Phe Ser Ile Val Thr Thr Tyr Phe
165 170 175

25 Pro Gly Tyr Gln Asp Phe Val Asn Lys Ile Arg Ser Thr Ile Glu Asn
180 185 190

Ser Phe Val Gly Trp Glu Leu Glu Val Leu Leu Asp Met Ser
195 200 205

30 Leu Asp Asp Gly Asp Ser Lys Ile Gln Asn Gln Leu Lys Lys Leu Gln
210 215 220

Ser Pro Ile Ile Leu Leu Tyr Cys Thr Lys Glu Glu Ala Thr Tyr Ile
35 225 230 235 240

Phe Glu Val Ala Asn Ser Val Gly Leu Thr Gly Tyr Gly Tyr Thr Trp
245 250 255

5 Ile Val Pro Ser Leu Val Ala Gly Asp Thr Asp Thr Val Pro Ala Glu
260 265 270

Phe Pro Thr Gly Leu Ile Ser Val Ser Tyr Asp Glu Trp Asp Tyr Gly
275 280 285

10 Leu Pro Pro Arg Val Arg Asp Gly Ile Ala Ile Ile Thr Thr Ala Ala
290 295 300

Ser Asp Met Leu Ser Glu His Ser Phe Ile Pro Glu Pro Lys Ser Ser
15 305 310 315 320

Cys Tyr Asn Thr His Glu Lys Arg Ile Tyr Gln Ser Asn Met Leu Asn
325 330 335

20 Arg Tyr Leu Ile Asn Val Thr Phe Glu Gly Arg Asn Leu Ser Phe Ser
340 345 350

Glu Asp Gly Tyr Gln Met His Pro Lys Leu Val Ile Ile Leu Leu Asn
355 360 365

25 Lys Glu Arg Lys Trp Glu Arg Val Gly Lys Trp Lys Asp Lys Ser Leu
370 375 380

Gln Met Lys Tyr Tyr Val Trp Pro Arg Met Cys Pro Glu Thr Glu Glu
385 390 395 400

Gln Glu Asp Asp His Leu Ser Ile Val Thr Leu Glu Glu Ala Pro Phe
405 410 415

35 Val Ile Val Glu Ser Val Asp Pro Leu Ser Gly Thr Cys Met Arg Asn

420 425 430
Thr Val Pro Cys Gln Lys Arg Ile Val Thr Glu Asn Lys Thr Asp Glu
435 440 445
5
Glu Pro Gly Tyr Ile Lys Lys Cys Cys Lys Gly Phe Cys Ile Asp Ile
450 455 460

Leu Lys Lys Ile Ser Lys Ser Val Lys Phe Thr Tyr Asp Leu Tyr Leu
10 465 470 475 480

Val Thr Asn Gly Lys His Gly Lys Lys Ile Asn Gly Thr Trp Asn Gly
485 490 495

15 Met Ile Gly Glu Val Val Met Lys Arg Ala Tyr Met Ala Val Gly Ser
500 505 510

Leu Thr Ile Asn Glu Glu Arg Ser Glu Val Val Asp Phe Ser Val Pro
515 520 525
20
Phe Ile Glu Thr Gly Ile Ser Val Met Val Ser Arg Ser Asn Gly Thr
530 535 540

Val Ser Pro Ser Ala Phe Leu Glu Pro Phe Ser Ala Asp Val Trp Val
25 545 550 555 560

Met Met Phe Val Met Leu Leu Ile Val Ser Ala Val Ala Val Phe Val
565 570 575

30 Phe Glu Tyr Phe Ser Pro Val Gly Tyr Asn Arg Cys Leu Ala Asp Gly
580 585 590

Arg Glu Pro Gly Gly Pro Ser Phe Thr Ile Gly Lys Ala Ile Trp Leu
595 600 605
35

Leu Trp Gly Leu Val Phe Asn Asn Ser Val Pro Val Gln Asn Pro Lys
 610 615 620

Gly Thr Thr Ser Lys Ile Met Val Ser Val Trp Ala Phe Phe Ala Val
5 625 630 635 640

Ile Phe Leu Ala Ser Tyr Thr Ala Asn Leu Ala Ala Phe Met Ile Gln
 645 650 655

10 Glu Glu Tyr Val Asp Gln Val Ser Gly Leu Ser Asp Lys Lys Phe Gln
 660 665 670

Arg Pro Asn Asp Phe Ser Pro Pro Phe Arg Phe Gly Thr Val Pro Asn
 675 680 685

15
 Gly Ser Thr Glu Arg Asn Ile Arg Asn Asn Tyr Ala Glu Met His Ala
 690 695 700

Tyr Met Gly Lys Phe Asn Gln Arg Gly Val Asp Asp Ala Leu Leu Ser
20 705 710 715 720

Leu Lys Thr Gly Lys Leu Asp Ala Phe Ile Tyr Asp Ala Ala Val Leu
 725 730 735

25 Asn Tyr Met Ala Gly Arg Asp Glu Gly Cys Lys Leu Val Thr Ile Gly
 740 745 750

Ser Gly Lys Val Phe Ala Ser Thr Gly Tyr Ile Ala Ile Gin Lys
 755 760 765

30
 Asp Ser Gly Trp Lys Arg Gln Val Asp Leu Ala Ile Leu Gln Leu Phe
 770 775 780

Gly Asp Gly Glu Met Glu Glu Leu Glu Ala Leu Trp Leu Thr Gly Ile
35 785 790 795 800

Cys His Asn Glu Lys Asn Glu Val Met Ser Ser Gln Leu Asp Ile Asp

805

810

815

5 Asn Met Ala Gly Val Phe Tyr Met Leu Gly Ala Ala Met Ala Leu Ser
820 825 830

Leu Ile Thr Phe Ile Cys Glu His Leu Phe Tyr Trp Gln Phe Arg His
835 840 845

10

Cys Phe Met Gly Val Cys Ser Gly Lys Pro Gly Met Val Phe Ser Ile
850 855 860

Ser Arg Gly Ile Tyr Ser Cys Ile His Gly Val Ala Ile Glu Glu Arg
15 865 870 875 880

Gln Ser Val Met Asn Ser Pro Thr Ala Thr Met Asn Asn Thr His Ser
885 890 895

20 Asn Ile Leu Arg Leu Leu Arg Thr Ala Lys Asn Met Ala Asn Leu Ser
900 905 910

Gly Val Asn Gly Ser Pro Gln Ser Ala Leu Asp Phe Ile Arg Arg Glu
915 920 925

25

Ser Ser Val Tyr Asp Ile Ser Glu His Arg Arg Ser Phe Thr His Ser
930 935 940

Asp Cys Lys Ser Tyr Asn Asn Pro Pro Cys Glu Glu Asn Leu Phe Ser
30 945 950 955 960

Asp Tyr Ile Ser Glu Val Glu Arg Thr Phe Gly Asn Leu Gln Leu Lys
965 970 975

35 Asp Ser Asn Val Tyr Gln Asp His Tyr His His His Arg Pro His

960

985

990

Ser Ile Gly Ser Ala Ser Ser Ile Asp Gly Leu Tyr Asp Cys Asp Asn

995

1000

1005

5

Pro Pro Phe Thr Thr Gln Ser Arg Ser Ile Ser Lys Lys Pro Leu Asp

1010

1015

1020

Ile Gly Leu Pro Ser Ser Lys His Ser Gln Leu Ser Asp Leu Tyr Gly

10

1025

1030

1035

1040

Lys Phe Ser Phe Lys Ser Asp Arg Tyr Ser Gly His Asp Asp Leu Ile

1045

1050

1055

15

Arg Ser Asp Val Ser Asp Ile Ser Thr His Thr Val Thr Tyr Gly Asn

1060

1065

1070

Ile Glu Gly Asn Ala Ala Lys Arg Arg Lys Gln Gln Tyr Lys Asp Ser

1075

1080

1085

20

Leu Lys Lys Arg Pro Ala Ser Ala Lys Ser Arg Arg Glu Phe Asp Glu

1090

1095

1100

Ile Glu Leu Ala Tyr Arg Arg Pro Pro Arg Ser Pro Asp His Lys

25

1105

1110

1115

1120

Arg Tyr Phe Arg Asp Lys Glu Gly Leu Arg Asp Phe Tyr Leu Asp Gln

1125

1130

1135

30

Phe Arg Thr Lys Glu Asn Ser Pro His Trp Glu His Val Asp Leu Thr

1140

1145

1150

Asp Ile Tyr Lys Glu Arg Ser Asp Asp Phe Lys Arg Asp Ser Ile Ser

1155

1160

1165

35

Gly Gly Gly Pro Cys Thr Asn Arg Ser His Ile Lys His Gly Thr Gly
 1170 1175 1180

Asp Lys His Gly Val Val Ser Gly Val Pro Ala Pro Trp Glu Lys Asn
5 1185 1190 1195 1200

Leu Thr Asn Val Glu Trp Glu Asp Arg Ser Gly Gly Asn Phe Cys Arg
 1205 1210 1215

10 Ser Cys Pro Ser Lys Leu His Asn Tyr Ser Thr Thr Val Thr Gly Gln
 1220 1225 1230

Asn Ser Gly Arg Gln Ala Cys Ile Arg Cys Glu Ala Cys Lys Ala
 1235 1240 1245

15

Gly Asn Leu Tyr Asp Ile Ser Glu Asp Asn Ser Leu Gln Glu Leu Asp
 1250 1255 1260

Gln Pro Ala Ala Pro Val Ala Val Thr Ser Asn Ala Ser Thr Thr Lys
20 1265 1270 1275 1280

Tyr Pro Gln Ser Pro Thr Asn Ser Lys Ala Gln Lys Lys Asn Arg Asn
 1285 1290 1295

25 Lys Leu Arg Arg Gln His Ser Tyr Asp Thr Phe Val Asp Leu Gln Lys
 1300 1305 1310

Glu Glu Ala Ala Leu Ala Pro Arg Ser Val Ser Leu Lys Asp Lys Gly
 1315 1320 1325

30

Arg Phe Met Asp Gly Ser Pro Tyr Ala His Met Phe Glu Met Ser Ala
 1330 1335 1340

Gly Glu Ser Thr Phe Ala Asn Asn Lys Ser Ser Val Pro Thr Ala Gly
35 1345 1350 1355 1360

His His His His Asn Asn Pro Gly Gly Gly Tyr Met Leu Ser Lys Ser

1365 1370 1375

5 Leu Tyr Pro Asp Arg Val Thr Gln Asn Pro Phe Ile Pro Thr Phe Gly

1380 1385 1390

Asp Asp Gln Cys Leu Leu His Gly Ser Lys Ser Tyr Phe Phe Arg Gln

1395 1400 1405

10

Pro Thr Val Ala Gly Ala Ser Lys Ala Arg Pro Asp Phe Arg Ala Leu

1410 1415 1420

Val Thr Asn Lys Pro Val Val Ser Ala Leu His Gly Ala Val Pro Ala

15 1425 1430 1435 1440

Arg Phe Gln Lys Asp Ile Cys Ile Gly Asn Gln Ser Asn Pro Cys Val

1445 1450 1455

20 Pro Asn Asn Thr Asn Pro Arg Ala Phe Asn Gly Ser Ser Asn Gly His

1460 1465 1470

Val Tyr Glu Lys Leu Ser Ser Ile Glu Ser Asp Val

1475 1480

25

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 4695 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: both

(D) TOPOLOGY: both

35 (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME KEY: CDS

(B) LOCATION: 485..4495

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

	CGAGAACACA GCGAGTGTGT GAGTCCTTCC CGCTCCAGCT CCTCCAAGCC	60
10	CGGGCACCCCT CGGGCGCAGC CTCCCCGAGC CTCCCTCGGC CACCGGTGTC TGGTGGGGGT	120
	GTTGCCTGGG TAGGTCTGGCC CGGGCCCCAG GGGTCTCTCG AGCGTCTGCC ATCTGCCCGA	180
	GAAACATGTG TGGGCACGTC CTGCTCTAGT CCAGGTGGCC GCAACCTTGG GGGAGAGACA	240
15	GGGCAGGACA GGACCAAGGT AAGAGGTAAG GAGGAGACGG CGCCAGGGAC AGACAGGAGG	300
	TCCCGGCTTG CGGTGTGCG CACCACACT GCGGGCGCCC CGGGGCTGC CGCCGACATC	360
20	GGCTCTCTGA GCGCTCTCG GAATCTTGGG GTCGCTGGAC GCGGGTTGC GGTCTGGCC	420
	CCCCCCCAT CGGGCAACA AACAGGGTC ATGAAAAGAG GCGGGCGGC GGGGCCCGCA	480
	GGCG ATG CGC GGC GCC GGT GGC CCC CGC GGC CCT CGG GGC CCC GCT AAG	529
25	Met Arg Gly Ala Gly Gly Pro Arg Gly Pro Arg Gly Pro Ala Cys	
	1 5 10 15	
	ATG CTG CTG CTG CTG GCG CTG GCC TGC GCC AGC CGT TTC CGG GAG GAG	577
	Met Leu Leu Leu Leu Ala Leu Ala Cys Ala Ser Pro Phe Pro Glu Glu	
30	20 25 30	
	GCG CCG CGG CGG GGC GGC GGT GGG CCC GGC CGG GGC CTC CGC GGG	625
	Ala Pro Gly Pro Gly Gly Ala Gly Gly Pro Gly Gly Leu Gly Gly	
	35 40 45	
35		

	GGG CGG CGG CTC AAC GTG GCG CTC GTG TTC TCG GGG CCC GCG TAC GCG	673		
	Ala Arg Pro Leu Asn Val Ala Leu Val Phe Ser Gly Pro Ala Tyr Ala			
	50	55	60	
5	GCC GAG GCG GCA CGC CTC GTG GGC CGG GCG GTG GCG GCG GCG GTG CGC AGC	721		
	Ala Glu Ala Ala Arg Leu Gly Pro Ala Val Ala Ala Val Arg Ser			
	65	70	75	
10	CGG GGC CTA GAC GTG CGG CGC CTG GCG CTG GTG CTC AAC GGC TCG GAC	769		
	Pro Gly Leu Asp Val Arg Pro Val Ala Leu Val Leu Asn Gly Ser Asp			
	80	85	90	95
	CGG CGC AGC CTC GTG CTG CAG CTC TGC GAC CTG CTG TCG GGG TTG CGC	817		
	Pro Arg Ser Leu Val Leu Gln Leu Cys Asp Leu Leu Ser Gly Leu Arg			
15	100	105	110	
	GTG CAC GGC GTG GTC TTC GAA GAC GAC TCG CGG CGC CGC GTC GCG	865		
	Val His Gly Val Val Phe Glu Asp Asp Ser Arg Ala Pro Ala Val Ala			
	115	120	125	
20	CCC ATC CTC GAC TTC CTG TCG GCG CAG ACC TCG CTC CGC ATC GTG TCC	913		
	Pro Ile Leu Asp Phe Leu Ser Ala Gln Thr Ser Leu Pro Ile Val Ser			
	130	135	140	
25	GAG CAC GGC GGC GCC GCG CTC GTG CTC ACG CGC AAG GAG AAG GGC TCC	961		
	Glu His Gly Gly Ala Ala Leu Val Leu Thr Pro Lys Glu Lys Gly Ser			
	145	150	155	
	ACC TTC CTC CAC CTG GGC TCT TCC CGG GAG CAA CAG CTT CAG GTC ATC	1009		
30	Thr Phe Leu His Leu Gly Ser Ser Pro Glu Gln Gln Leu Gln Val Ile			
	160	165	170	175
	TTT GAG GTG CTG GAG GAG TAT GAC TGG ACG TCC TTT GTA GCG GTG ACC	1057		
	Phe Glu Val Leu Glu Glu Tyr Asp Trp Thr Ser Phe Val Ala Val Thr			
35	180	185	190	

	ACT CGT GCC CCT GGC CAC CGG GCC TTC CTG TCC TAC ATT GAG GTG CTG	1195	
	Thr Arg Ala Pro Gly His Arg Ala Phe Leu Ser Tyr Ile Glu Val Leu		
	195	200	205
5			
	ACT GAC GGC ACT CTG GTG GGC TGG GAG CAC CGC GGA GCG CTG ACG CTG	1153	
	Thr Asp Gly Ser Leu Val Gly Trp Glu His Arg Gly Ala Leu Thr Leu		
	210	215	220
10	GAC CCT GGG GCG GGC GAG GCC GTG CTC AGT GCC CAG CTC CGC AGT GTC	1201	
	Asp Pro Gly Ala Gly Glu Ala Val Leu Ser Ala Gln Leu Arg Ser Val		
	225	230	235
	AGC GCG CAG ATC CGC CTG CTC TTC TGC GCC CGA GAG GAG GCC GAG CCC	1249	
15	Ser Ala Gln Ile Arg Leu Leu Phe Cys Ala Arg Glu Glu Ala Glu Pro		
	240	245	250
	GTG TTC CGC GCA GCT GAG GAG GCT GGC CTC ACT GGA TCT GGC TAC GTC	1297	
	Val Phe Arg Ala Ala Glu Glu Ala Gly Leu Thr Gly Ser Gly Tyr Val		
20	260	265	270
	TGG TTC ATG GTG GGG CCC CAG CTG GCT GGA GGC GGG GGC TCT GGG GCC	1345	
	Trp Phe Met Val Gly Pro Gln Leu Ala Gly Gly Ser Gly Ala		
	275	280	285
25			
	CCT GGT GAG CCC CCT CTT CTG CCA GGA GGC GCC CCC CTG CCT GCC GGG	1393	
	Pro Gly Glu Pro Pro Leu Leu Pro Gly Gly Ala Pro Leu Pro Ala Gly		
	290	295	300
30	CTG TTT GCA GTG CGC TCG GCT GGC TGG CGG GAT GAC CTG GGT CGG CGA	1441	
	Leu Phe Ala Val Arg Ser Ala Gly Trp Arg Asp Asp Leu Ala Arg Arg		
	305	310	315
	GTG GCA GGT GGC GTG GCD GTA GTG GCC AGA GGT GCC CAG GGC CTG CTG	1489	
35	Val Ala Ala Gly Val Ala Val Ala Arg Gly Ala Gln Ala Leu Leu		

	320	325	330	335	
	CGT GAT TAT GGT TTC CTT CCT GAG CTC GGC CAC GAC TGT CGC GCC CAG				1537
	Arg Asp Tyr Gly Phe Leu Pro Glu Leu Gly His Asp Cys Arg Ala Gln				
5	340	345	350		
	AAC CGC ACC CAC CGC GGG GAG AGT CTG CAT AGG TAC TTC ATG AAC ATC				1585
	Asn Arg Thr His Arg Gly Glu Ser Leu His Arg Tyr Phe Met Asn Ile				
	355	360	365		
10					
	ACG TGG GAT AAC CGG GAT TAC TCC TTC AAT GAG GAC GGC TTC CTA GTG				1633
	Thr Trp Asp Asn Arg Asp Tyr Ser Phe Asn Glu Asp Gly Phe Leu Val				
	370	375	380		
15					
	AAC CCC TCC CTG GTG GTC ATC TCC CTC ACC AGA GAC AGG ACG TGG GAG				1681
	Asn Pro Ser Leu Val Val Ile Ser Leu Thr Arg Asp Arg Thr Trp Glu				
	385	390	395		
	GTG GTG GGC AGC TGG GAG CAG CAG ACG CTC CGC CTC AAG TAC CCG CTG				1729
20	Val Val Gly Ser Trp Glu Gln Gln Thr Leu Arg Leu Lys Tyr Pro Leu				
	400	405	410	415	
	Trp Ser Arg Tyr Gly Arg Phe Leu Gln Pro Val Asp Asp Thr Gln His				
25	420	425	430		
	CTC GCG GTG GGC ACG CTG GAG GAA AGG CCG TTT GTC ATC GTG GAG CCT				1825
	Leu Ala Val Ala Thr Leu Glu Glu Arg Pro Phe Val Ile Val Glu Pro				
	435	440	445		
30					
	GCA GAC CCT ATC AGC GGC ACC TGC ATC CGA GAC TCC GTC CCC TGC CGG				1873
	Ala Asp Pro Ile Ser Gly Thr Cys Ile Arg Asp Ser Val Pro Cys Arg				
	450	455	460		
35					
	AGC CAG CTC AAC CGA ACC CAT AGC CCT CCA CGG GAT GCC CCC CGC CCG				1921

	Ser Gln Leu Asn Arg Thr His Ser Pro Pro Pro Asp Ala Pro Arg Pro		
	465	470	475
	GAA AAG CGC TGC TGC AAG GGT TTC TGC ATC GAC ATT CTG AAG CGG CTG		1969
5	Glu Lys Arg Cys Cys Lys Gly Phe Cys Ile Asp Ile Leu Lys Arg Leu		
	480	485	490
	GCG CAT ACC ATC GGC TTC AGC TAC GAC CTC TAC CTG GTC ACC AAT GGC		2017
	Ala His Thr Ile Gly Phe Ser Tyr Asp Leu Tyr Leu Val Thr Asn Gly		
10	500	505	510
	AAG CAC GGA AAG AAG ATC GAT GGC GTC TGG AAC GGC ATG ATC GGG GAG		2065
	Lys His Gly Lys Lys Ile Asp Gly Val Trp Asn Gly Met Ile Gly Glu		
	515	520	525
15			
	GTG TTC TAC CAG CGC GCA GAC ATG GCC ATC GGC TCC CTC ACC ATC AAC		2113
	Val Phe Tyr Gln Arg Ala Asp Met Ala Ile Gly Ser Leu Thr Ile Asn		
	530	535	540
20	GAG GAG CGC TCC GAG ATC GTG GAC TTC TCC GTC CCC TTC GTG GAG ACC		2161
	Glu Glu Arg Ser Glu Ile Val Asp Phe Ser Val Pro Phe Val Glu Thr		
	545	550	555
	GGC ATC AGC GTC ATG GTG GCG CGC AGC AAT GGC ACG GTG TCC CCC TCG		2209
25	Gly Ile Ser Val Met Val Ala Arg Ser Asn Gly Thr Val Ser Pro Ser		
	560	565	570
	GCC TTC CTC GAG CCC TAC AGC CCC GCC GTG TGG GTG ATG ATG TTC GTC		2257
	Ala Phe Leu Glu Pro Tyr Ser Pro Ala Val Trp Val Met Met Phe Val		
30	580	585	590
	ATG TGC CTC ACT GTG GTC GCC GTC ACT GTT TTC ATC TTC GAG TAC CTC		2305
	Met Cys Leu Thr Val Val Ala Val Thr Val Phe Ile Phe Glu Tyr Leu		
	595	600	605

	AGT CCT GTT GGT TAC AAC CGC AGC CTG GCC ACG GGC AAG CGC CCT GGC		2353
	Ser Pro Val Gly Tyr Asn Arg Ser Leu Ala Thr Gly Lys Arg Pro Gly		
	610	615	620
5	GST TCA ACC TTC ACC ATT GGG AAA TCC ATC TGG CTG CTC TGG GGC CTG		2401
	Gly Ser Thr Phe Thr Ile Gly Lys Ser Ile Trp Leu Leu Trp Ala Leu		
	625	630	635
	GTG TTC AAT AAT TCG GTG CCC GTG GAG AAC CGC CGG GGA ACC ACC AGC		2449
10	Val Phe Asn Asn Ser Val Pro Val Glu Asn Pro Arg Gly Thr Thr Ser		
	640	645	650
	AAA ATC ATG GTG CTG GTG TGG GCC TTC TPC GGC GTC ATC TTC CTC GCC		2497
	Lys Ile Met Val Leu Val Trp Ala Phe Phe Ala Val Ile Phe Leu Ala		
15	660	665	670
	AGC TAC ACA GCC AAC CTG GCC GCC TTC ATG ATC CAG GAG GAG TAC GTG		2545
	Ser Tyr Thr Ala Asn Leu Ala Ala Phe Met Ile Gln Glu Glu Tyr Val		
	675	680	685
20	GAT ACT GTG TCT GGG CTC AGT GAC CGC AAG TTC CAG AGG CCC CAG GAG		2593
	Asp Thr Val Ser Gly Leu Ser Asp Arg Lys Phe Gln Arg Pro Gln Glu		
	690	695	700
25	CAG TAC CCG CCC CTG AAG TTT GGG ACC GTG CCC AAC GGC TCC AGG SAG		2641
	Gin Tyr Pro Pro Leu Lys Phe Gly Thr Val Pro Asn Gly Ser Thr Glu		
	705	710	715
	AAG AAC ATC CGC AGC AAC TAT CCC GAC ATG CAC AGC TAC ATG GTG CGC		2689
30	Lys Asn Ile Arg Ser Asn Tyr Pro Asp Met His Ser Tyr Met Val Arg		
	720	725	730
	TAC AAC CAG CCC CGG GTA GAG GAA GCG CTC ACT CAG CTC AAG GCA GGG		2737
	Tyr Asn Gln Pro Arg Val Glu Glu Ala Leu Thr Gln Leu Lys Ala Gly		
35	740	745	750

	AAG CTG GAC GCG TTC ATC TAC GAT GCT GCA GTG CTC AAT TAC ATG GCC	2785
	Lys Leu Asp Ala Phe Ile Tyr Asp Ala Ala Val Leu Asn Tyr Met Ala	
	755 760 765	
5		
	CGC AAG GAC GAG GGC TGC AAG CTT GTC ACC ATC GGC TCC GGC AAG GTC	2833
	Arg Lys Asp Glu Gly Cys Lys Leu Val Thr Ile Gly Ser Gly Lys Val	
	770 775 780	
10	TTC GCC ACG ACA GGC TAT GGC ATC GCC CTG CAC AAG GGC TCC CGC TGG	2881
	Phe Ala Thr Thr Gly Tyr Gly Ile Ala Leu His Lys Gly Ser Arg Trp	
	785 790 795	
	AAG CGG CCC ATC GAC CTG GCG TTG CTG CAG TTC CTG GGG GAT GAT GAG	2929
15	Lys Arg Pro Ile Asp Leu Ala Leu Leu Gln Phe Leu Gly Asp Asp Glu	
	800 805 810 815	
	ATC GAG ATG CTG GAG CGG CTG TGG CTC TCT GGG ATC TGC CAC AAT GAC	2977
	Ile Glu Met Leu Glu Arg Leu Trp Leu Ser Gly Ile Cys His Asn Asp	
20	820 825 830	
	AAA ATC GAG GTG ATG AGC AGC AAG CTG GAC ATC GAC AAC ATG GCG GGC	3025
	Lys Ile Glu Val Met Ser Ser Lys Leu Asp Ile Asp Asn Met Ala Gly	
	835 840 845	
25		
	GTC TTC TAC ATG CTC CTG GTG GCC ATG GGC CTG TCC CTG CTG GTC TTC	3073
	Val Phe Tyr Met Leu Leu Val Ala Met Gly Leu Ser Leu Leu Val Phe	
	850 855 860	
30	GCC TGG GAG CAC CTG GTG TAC TGG CGC CTG CGG CAC TGC CTG GGG CCC	3121
	Ala Trp Glu His Leu Val Tyr Trp Arg Leu Arg His Cys Leu Gly Pro	
	865 870 875	
	AGC CAC CGC ATG GAC TTC CTG CTG GCC TTC TCC AGG GGC ATG TAC AGC	3169
35	Thr His Arg Met Asp Phe Leu Leu Ala Phe Ser Arg Gly Met Tyr Ser	

	881	885	891	895	
	TGC TGC AGC GCT GAG GCC GCG CCA CCG CCC GCC AAG CCC CGG CCG CGG				3217
	Cys Cys Ser Ala Glu Ala Ala Pro Pro Pro Ala Lys Pro Pro Pro Pro				
5	900	905	910		
	CCA CAG CCC CTG CCC AGC CCC GCG TAC CCC GCG CGG CGG CGG GCT CCC				3265
	Pro Gln Pro Leu Pro Ser Pro Ala Tyr Pro Ala Pro Gly Pro Ala Pro				
	915	920	925		
10					
	GGG CCC GCA CCT TTC GTC CCC CGC GAG CGC GCC TCA GTG GCC CGC TGG				3313
	Gly Pro Ala Pro Phe Val Pro Arg Glu Arg Ala Ser Val Ala Arg Trp				
	930	935	940		
15					
	GGC CGG CCC AAG GGC GCG GGG CGG CGG GGG GGC GCG GGC CTG GCC GAC				3361
	Arg Arg Pro Lys Gly Ala Gly Pro Pro Gly Gly Ala Gly Leu Ala Asp				
	945	950	955		
	GGC TTC CAC CGC TAC TAC GGC CCC ATC GAG CGG CAG GGC CTA GGC CTC				3409
20	Gly Phe His Arg Tyr Tyr Gly Pro Ile Glu Pro Gln Gly Leu Gly Leu				
	960	965	970	975	
	GGC CTG GGC GAA GCG CGC GCG GCA CGG CGG GGC GCA GCC GGG CGC CGG				3457
	Gly Leu Gly Glu Ala Arg Ala Ala Pro Arg Gly Ala Ala Gly Arg Pro				
25	980	985	990		
	CTG TCC CGG CGG GCC GCT CAG CCC CGG CAG AAG CGG CGG CGG TCC TAT				3505
	Leu Ser Pro Pro Ala Ala Gln Pro Pro Gln Lys Pro Pro Ala Ser Tyr				
	995	1000	1005		
30					
	TTC GCC ATC GTA CGC GAC AAG GAG CCA GCC GAG CCC CCC GCG GGC GGC				3553
	Phe Ala Ile Val Arg Asp Lys Glu Pro Ala Gln Pro Pro Ala Gly Ala				
	1010	1015	1020		
35	TTC CGG GGC TTC CGG CGG CGG CGG CGG CGG CGG CGG CGG ACC				3601

Phe Pro Gly Phe Pro Ser Pro Pro Ala Pro Pro Ala Ala Ala Thr
 1025 1030 1035

GCC GTC GGG CGG CCA CTC TGC CGC TTG GCC TTC GAG GAC GAG AGC CCG 3649
5 Ala Val Gly Pro Pro Leu Cys Arg Leu Ala Phe Glu Asp Glu Ser Pro
 1040 1045 1050 1055

CCG CGG CCC GCG CGG TGG CGG CCG TCG GAC CGC GAG AGC CAA CCC CTG 3697
 Pro Ala Pro Ala Arg Trp Pro Arg Ser Asp Pro Glu Ser Gln Pro Leu
10 1060 1065 1070

CTG GGG CCA GGC GCG GGC GGC GCG GGG GGC ACG GGG GGC GCA GGC GGA 3745
 Leu Gly Pro Gly Ala Gly Gly Ala Gly Thr Gly Gly Ala Gly Gly
 1075 1080 1085

15

GGA GCC CGG GCC GCT CGG CCC CGG TGC TTC GCC GCG CGG CCC CGG TGC 3793
 Gly Ala Pro Ala Ala Pro Pro Pro Cys Phe Ala Ala Pro Pro Pro Cys
 1090 1095 1100

20 TTT TAC CTC GAT GTC GAC CAG TCG CCG TCG GAC TCG GAG GAC TCG GAG 3841
 Phe Tyr Leu Asp Val Asp Gln Ser Pro Ser Asp Ser Glu Asp Ser Glu
 1105 1110 1115

AGC CTG GCC GGC GCG TCC CTG GCC GGC CTG GAT CCC TGG TGG TTC GCC 3889
25 Ser Leu Ala Gly Ala Ser Leu Ala Gly Leu Asp Pro Trp Trp Phe Ala
 1120 1125 1130 1135

GAC TTC CCT TAC CGG TAT GCC GAT CGC CTC GGG CGG CCC GCG GCA CGC 3937
 Asp Phe Pro Tyr Pro Tyr Ala Asp Arg Leu Gly Xaa Pro Ala Ala Arg
30 1140 1145 1150

TAT GGA TTG GTC GAC AAA CTA GGG GGC TGG CTC GCC GGG AGC TGG GAC 3965
 Tyr Gly Leu Val Asp Lys Leu Gly Gly Trp Leu Ala Gly Ser Trp Asp
 1155 1160 1165

	TAC CTG CCT CCS CGC AGC GGT CGG GCC GCC TGG CAC TGT CGG CAC TGC	4033		
	Tyr Leu Pro Xaa Arg Ser Gly Arg Ala Ala Trp His Cys Arg His Cys			
	1170	1175	1180	
5	GCC AGC CTG GAG CTG CTT CCG CGG CGG CGC CAT CTC AGC TGC TCG CAC	4081		
	Ala Ser Leu Glu Leu Leu Pro Pro Pro Arg His Leu Ser Cys Ser His			
	1185	1190	1195	
	GAT GGC CTG GAC GGC GGC TGG TGG GCG CCA CGG CCT CCA CCC TGG GGC	4129		
10	Asp Gly Leu Asp Gly Gly Trp Trp Ala Pro Pro Pro Pro Pro Trp Ala			
	1200	1205	1210	1215
	GCC GGG CCC CTG CCC CGA CGC CGG GCC CGC TGC GGG TGC CGG CGG TCG	4177		
	Ala Gly Pro Leu Pro Arg Arg Arg Ala Arg Cys Gly Cys Pro Arg Ser			
15	1220	1225	1230	
	CAC CCG CAC CGC CGG CGC TCG CAC CGC ACG CCC GCC GCT GCC GCG	4225		
	His Pro His Arg Pro Arg Ala Ser His Arg Thr Pro Ala Ala Ala			
	1235	1240	1245	
20	CCC CAC CAC CAC AGG CAC CGG CGC GCC GCT GGG GGC TGG GAC CTC CGG	4273		
	Pro His His Arg His Arg Arg Ala Ala Gly Gly Trp Asp Leu Pro			
	1250	1255	1260	
25	CCG CCC GCG CCC ACC TCG CGC TCG CTC GAG GAC CTC AGC TCG TGC CCT	4321		
	Pro Pro Ala Pro Thr Ser Arg Ser Leu Glu Asp Leu Ser Ser Cys Pro			
	1265	1270	1275	
	CGC GCC GCC CCT CGG CGC AGG CTT ACC GGG CCC TCC CGC CAC GCT CGC	4369		
30	Arg Ala Ala Pro Ala Arg Arg Leu Thr Gly Pro Ser Arg His Ala Arg			
	1280	1285	1290	1295
	AGG TGT CGG CAC GCC CGC CAC TGG GGG CGG CGG CTG CCT ACA GCT TCC	4417		
	Arg Cys Pro His Ala Ala His Trp Gly Pro Pro Leu Pro Thr Ala Ser			
35	1300	1305	1310	

CAC CGG AGA CAC CGG GGC GGG GAC CTG GGC ACC CGC AGG GGC TCG GCG 4465
 His Arg Arg His Arg Gly Gly Asp Leu Gly Thr Arg Arg Gly Ser Ala

1315 1320 1325

5

CAC TTC TCT AGC CTC GAG TCC GAG GTA TGACGGGGCC CCGGGGGCCC 4512
 His Phe Ser Ser Leu Glu Ser Glu Val

1330 1335

10 CACCGCCCCC TTGGTCAGCG CAGGCCACGG CCCGAGGGGG CGCCCGCAGT GGACAGGACC 4572

CGCGTGGGTT GGGAAAGGAAA GCAGTGAAAC TGGCCGGACC CGCCCTGGAG CAGCGTCCTG 4632

CGCCCCCTGG TTCTGGAGGA ACCGCAAGCC GGAGAGGATT TGGTCCCTCA ACTATCACCC 4692

15

AGG 4695

(2) INFORMATION FOR SEQ ID NO:16:

20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1336 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

25

- (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

30 Met Arg Gly Ala Gly Gly Pro Arg Gly Pro Arg Gly Pro Ala Lys Met

1 5 10 15

Ieu Leu Leu Leu Ala Leu Ala Sys Ala Ser Pro Phe Pro Glu Glu Ala

20 25 30

35

Pro Gly Pro Gly Gly Ala Gly Gly Pro Gly Gly Leu Gly Gly Ala
 35 40 45

Arg Pro Leu Asn Val Ala Leu Val Phe Ser Gly Pro Ala Tyr Ala Ala
5 50 55 60

Glu Ala Ala Arg Leu Gly Pro Ala Val Ala Ala Ala Val Arg Ser Pro
 65 70 75 80

10 Gly Leu Asp Val Arg Pro Val Ala Leu Val Leu Asn Gly Ser Asp Pro
 85 90 95

Arg Ser Leu Val Leu Gln Leu Cys Asp Leu Leu Ser Gly Leu Arg Val
 100 105 110

15
 His Gly Val Val Phe Glu Asp Asp Ser Arg Ala Pro Ala Val Ala Pro
 115 120 125

Ile Leu Asp Phe Leu Ser Ala Gln Thr Ser Leu Pro Ile Val Ser Glu
20 130 135 140

His Gly Gly Ala Ala Leu Val Leu Thr Pro Lys Glu Lys Gly Ser Thr
 145 150 155 160

25 Phe Leu His Leu Gly Ser Ser Pro Glu Gln Gln Leu Gln Val Ile Phe
 165 170 175

Glu Val Leu Glu Glu Tyr Asp Trp Thr Ser Phe Val Ala Val Thr Thr
 180 185 190

30
 Arg Ala Pro Gly His Arg Ala Phe Leu Ser Tyr Ile Glu Val Leu Thr
 195 200 205

Asp Gly Ser Leu Val Gly Trp Glu His Arg Gly Ala Leu Thr Leu Asp
35 210 215 220

Pro Gly Ala Gly Glu Ala Val Leu Ser Ala Gln Leu Arg Ser Val Ser
 225 230 235 240

5 Ala Gin Ile Arg Leu Leu Phe Cys Ala Arg Glu Glu Ala Glu Pro Val
 245 250 255

Phe Arg Ala Ala Glu Glu Ala Gly Leu Thr Gly Ser Gly Tyr Val Trp
 260 265 270

10
 Phe Met Val Gly Pro Gln Leu Ala Gly Gly Gly Ser Gly Ala Pro
 275 280 285

Gly Glu Pro Pro Leu Leu Pro Gly Gly Ala Pro Leu Pro Ala Gly Leu
15 290 295 300

Phe Ala Val Arg Ser Ala Gly Trp Arg Asp Asp Leu Ala Arg Arg Val
 305 310 315 320

20 Ala Ala Gly Val Ala Val Val Ala Arg Gly Ala Gln Ala Leu Leu Arg
 325 330 335

Asp Tyr Gly Phe Leu Pro Glu Leu Gly His Asp Cys Arg Ala Gln Asn
 340 345 350

25
 Arg Thr His Arg Gly Glu Ser Leu His Arg Tyr Phe Met Asn Ile Thr
 355 360 365

Trp Asp Asn Arg Asp Tyr Ser Phe Asn Glu Asp Gly Phe Leu Val Asn
30 370 375 380

Pro Ser Leu Val Val Ile Ser Leu Thr Arg Asp Arg Arg Thr Trp Glu Val
 385 390 395 400

35 Val Gly Ser Trp Glu Gln Gln Thr Leu Arg Leu Lys Tyr Pro Leu Trp

	405	410	415
	Ser Arg Tyr Gly Arg Phe Leu Gln Pro Val Asp Asp Thr Gln His Leu		
	420	425	430
5	Ala Val Ala Thr Leu Glu Glu Arg Pro Phe Val Ile Val Glu Pro Ala		
	435	440	445
	Asp Pro Ile Ser Gly Thr Cys Ile Arg Asp Ser Val Pro Cys Arg Ser		
10	450	455	460
	Gln Leu Asn Arg Thr His Ser Pro Pro Pro Asp Ala Pro Arg Pro Glu		
	465	470	475
	480		
15	Lys Arg Cys Cys Lys Gly Phe Cys Ile Asp Ile Leu Lys Arg Leu Ala		
	485	490	495
	His Thr Ile Gly Phe Ser Tyr Asp Leu Tyr Leu Val Thr Asn Gly Lys		
	500	505	510
20	515		
	His Gly Lys Lys Ile Asp Gly Val Trp Asn Gly Met Ile Gly Glu Val		
	520	525	
	Phe Tyr Gln Arg Ala Asp Met Ala Ile Gly Ser Leu Thr Ile Asn Glu		
25	530	535	540
	545		
	550		
	555	560	
	Glu Arg Ser Glu Ile Val Asp Ser Val Pro Phe Val Glu Thr Gly		
30	Ile Ser Val Met Val Ala Arg Ser Asn Gly Thr Val Ser Pro Ser Ala		
	565	570	575
	580		
	585	590	
35	Phe Leu Glu Pro Tyr Ser Pro Ala Val Trp Val Met Met Phe Val Met		

Cys Leu Thr Val Val Ala Val Thr Val Phe Ile Phe Glu Tyr Leu Ser
 595 600 605

5 Pro Val Gly Tyr Asn Arg Ser Leu Ala Thr Gly Lys Arg Pro Gly Gly
 610 615 620

Ser Thr Phe Thr Ile Gly Lys Ser Ile Trp Leu Leu Trp Ala Leu Val
 625 630 635 640

10 Phe Asn Asn Ser Val Pro Val Glu Asn Pro Arg Gly Thr Thr Ser Lys
 645 650 655

Ile Met Val Leu Val Trp Ala Phe Phe Ala Val Ile Phe Leu Ala Ser
 660 665 670

15 Tyr Thr Ala Asn Leu Ala Ala Phe Met Ile Gln Glu Glu Tyr Val Asp
 675 680 685

20 Thr Val Ser Gly Leu Ser Asp Arg Lys Phe Gln Arg Pro Gln Glu Gln
 690 695 700

Tyr Pro Pro Leu Lys Phe Gly Thr Val Pro Asn Gly Ser Thr Glu Lys
 705 710 715 720

25 Asn Ile Arg Ser Asn Tyr Pro Asp Met His Ser Tyr Met Val Arg Tyr
 725 730 735

Asn Gln Pro Arg Val Glu Glu Ala Leu Thr Gln Leu Lys Ala Gly Lys
 740 745 750

30 Leu Asp Ala Phe Ile Tyr Asp Ala Ala Val Leu Asn Tyr Met Ala Arg
 755 760 765

Lys Asp Glu Gly Cys Lys Leu Val Thr Ile Gly Ser Gly Lys Val Phe
 770 775 780

Ala Thr Thr Gly Tyr Gly Ile Ala Leu His Lys Gly Ser Arg Trp Lys
 785 790 795 800

5 Arg Pro Ile Asp Leu Ala Leu Gln Phe Leu Gly Asp Asp Glu Ile
 805 810 815

Glu Met Leu Glu Arg Leu Trp Leu Ser Gly Ile Cys His Asn Asp Lys
 820 825 830

10
 Ile Glu Val Met Ser Ser Lys Leu Asp Ile Asp Asn Met Ala Gly Val
 835 840 845

Phe Tyr Met Leu Leu Val Ala Met Gly Leu Ser Leu Leu Val Phe Ala
15 850 855 860

Trp Glu His Leu Val Tyr Trp Arg Leu Arg His Cys Leu Gly Pro Thr
 865 870 875 880

20 His Arg Met Asp Phe Leu Leu Ala Phe Ser Arg Gly Met Tyr Ser Cys
 885 890 895

Cys Ser Ala Glu Ala Ala Pro Pro Pro Ala Lys Pro Pro Pro Pro
 900 905 910

25
 Gln Pro Leu Pro Ser Pro Ala Tyr Pro Ala Pro Gly Pro Ala Pro Gly
 915 920 925

Pro Ala Pro Phe Val Pro Arg Glu Arg Ala Ser Val Ala Arg Trp Arg
30 930 935 940

Arg Pro Lys Gly Ala Gly Pro Pro Gly Gly Ala Gly Leu Ala Asp Gly
 945 950 955 960

35 Phe His Arg Tyr Tyr Gly Pro Ile Glu Pro Gln Gly Leu Gly Leu Gly

965

971

975

Leu Gly Glu Ala Arg Ala Ala Pro Arg Gly Ala Ala Gly Arg Pro Leu

980

985

990

5

Ser Pro Pro Ala Ala Gln Pro Pro Gln Lys Pro Pro Ala Ser Tyr Phe

995

1000

1005

Ala Ile Val Arg Asp Lys Glu Pro Ala Glu Pro Pro Ala Gly Ala Phe

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1010

1015

1020

Pro Gly Phe Pro Ser Pro Pro Ala Pro Pro Ala Ala Ala Thr Ala

1025

1030

1035

1040

15

Val Gly Pro Pro Leu Cys Arg Leu Ala Phe Glu Asp Glu Ser Pro Pro

1045

1050

1055

Ala Pro Ala Arg Trp Pro Arg Ser Asp Pro Glu Ser Gln Pro Leu Leu

1060

1065

1070

20

Gly Pro Gly Ala Gly Gly Ala Gly Thr Gly Gly Ala Gly Gly Gly

1075

1080

1085

Ala Pro Ala Ala Pro Pro Pro Cys Phe Ala Ala Pro Pro Pro Cys Phe

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1090

1095

1100

Tyr Leu Asp Val Asp Gln Ser Pro Ser Asp Ser Glu Asp Ser Glu Ser

1105

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1115

1120

30

Leu Ala Gly Ala Ser Leu Ala Gly Leu Asp Pro Trp Trp Phe Ala Asp

1125

1130

1135

Phe Pro Tyr Pro Tyr Ala Asp Arg Leu Gly Xaa Pro Ala Ala Arg Tyr

1140

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1150

35

Gly Leu Val Asp Lys Leu Gly Gly Trp Leu Ala Gly Ser Trp Asp Tyr

1155

1160

1165

Leu Pro Xaa Arg Ser Gly Arg Ala Ala Trp His Cys Arg His Cys Ala

5

1170

1175

1180

Ser Leu Glu Leu Leu Pro Pro Pro Arg His Leu Ser Cys Ser His Asp

1185

1190

1195

1200

10

Gly Leu Asp Gly Gly Trp Trp Ala Pro Pro Pro Pro Pro Trp Ala Ala

1205

1210

1215

Gly Pro Leu Pro Arg Arg Ala Arg Cys Gly Cys Pro Arg Ser His

1220

1225

1230

15

Pro His Arg Pro Arg Ala Ser His Arg Thr Pro Ala Ala Ala Pro

1235

1240

1245

His His His Arg His Arg Arg Ala Ala Gly Gly Trp Asp Leu Pro Pro

20

1250

1255

1260

Pro Ala Pro Thr Ser Arg Ser Leu Glu Asp Leu Ser Ser Cys Pro Arg

1265

1270

1275

1280

25

Ala Ala Pro Ala Arg Arg Leu Thr Gly Pro Ser Arg His Ala Arg Arg

1285

1290

1295

Cys Pro His Ala Ala His Trp Gly Pro Pro Leu Pro Thr Ala Ser His

1300

1305

1310

30

Arg Arg His Arg Gly Gly Asp Leu Gly Thr Arg Arg Gly Ser Ala His

1315

1320

1325

Phe Ser Ser Leu Glu Ser Glu Val

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1330

1335

(2) INFORMATION FOR SEQ ID NO:17:

5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: both

10 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

15 GGGTGGCGGC CGCAGAGCAC CTCCACCATC TCCTTGTCT ACTCCAAGAT CTGGCCCTAG 60

TCCATGTTTG C 71

20 (2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TGGTGGTCGG CAACCTGTAG GACTTGGTTC TGGAGGAGGA TCTGGTGTAG GCAAACATGG 60

ACTAGGGCCA G 71

35

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

10 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GTTGGGGACC ACCAGATGGA GGTAGAGCTG CACTTGTACG AAGAGCTCCA CAACCACCTG 60

(2) INFORMATION FOR SEQ ID NO:20:

(iii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

30 CCTGAGACGT CAGACAAAGG AGGCCAGGT GTAGGTGGTC TACCAGGTGG TTGTGGAGCT 62

35

(2) INFORMATION FOR SEQ ID NO:21:

(1) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 195 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: both
 (D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CCGCAGAGCA CCTCCACCAT CTCCTTGTC TACTCCAAGA TCTGGCCCTA GTCCATGTTT 60

15

GCCTACACCA GATCCTCCTC CAGAACCAAG TCCTACAGGT TGGGGACCAC CAGATGGAGG 120

TAGAGCTGCA CTTGTACGAA GAGCTCCACA ACCACCTGGT AGACCACCTA CACCTGGCC 180

TCCTTTGTCT GACGT 195

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